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SIMULTANEOUS ABO AND Rh GROUPINGS ON CARDS

IN THE LABORATORY OR AT THE BEDSIDE

By KNUD ELDON

The value of a method of blood grouping depends upon its reliability, its technical and educational demands on the personnel and its speed of execution. The possible sources of error with any new method must be estimated from a knowledge of those inherent in other methods. Whether or not the method is suitable for specialized blood grouping laboratories only, or whether it can be used in the local laboratory or even at the bedside, must also be taken into consideration.

Centralized ABO and Rh Groupings.

In specialized laboratories, performing dual tests, the percentage of errors can be kept at a minimum. There is still, however, the risk of identity errors in obtaining the specimens and recording the results. Furthermore, it may be inconvenient to wait hours (or days) for an Rh result; and in general the size of the donor corps does not allow the administration of Rh-negative blood for all emergency transfusions. The blood groupings, therefore, should preferably be carried out on the spot.

ABO Groupings in Hospital Laboratories.

In general hospital laboratories, the ABO groupings are mostly performed following the well-known slide technique. This examination of the cells is not regularly followed by an additional examination of the serum against known A and B blood cells (back-typing). The most important of the possible sources of error is the risk of interchanging (19) either the test sera, the specimens or the results. In addition, errors may be made as a result of misunderstandings and deviations from the directions. In rarer cases, false positive reactions appear as a result of auto-agglutinins, rouleaux formation, infected test sera or specimens, or special characteristics in certain blood samples from the umbilical cord; and false negative reactions may be due to inhibitory prozones or impurities, e. g. traces of detergents on the slides. The well-known danger of missing the weak A_2, especially in the case of A_2B formerly played a more important part, as noted in Salber's survey in 1951 (13) of the follow-ups made by various authors, of mass blood groupings without back-typing. The average percentage of errors found here in the ABO groupings carried out by trained workers amounts to 0.5—1.0 %, by untrained to 4—10 %. For individual groupings, according to Moreau (9) back-typing should bring down the ABO errors of trained workers from 1:200 to 1:40,000. This does not work out in practice, because the back-typing, regardless of its excellence, cannot give as certain results as investigation of the blood cells and, furthermore, cannot guard against mix-ups or clerical errors outside the laboratory.

Rh Groupings in Hospital Laboratories.

In hospital laboratories, Rh groupings can be made with Diamond and Abelson's (2) slide technique and special heating apparatus. The possibilities for mix-ups and other sources of error are on the whole the same as with ABO

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groupings; however, rouleaux formation and auto-agglutinins have increased influence on the reactions.

Blood Groupings at the Bedside.

Methods for routine individual groupings outside the laboratories must be rapid and simple and should not require special installations or complicated apparatus. Thus ABO groupings can be carried out at the patients bed with the ordinary slide technique. It is not particularly practical, however, to use liquid test sera at the bedside, and in return for security against interchanging the specimens, the risk of interchanging the test sera is enlarged by such separate determinations.

If it were possible to use test sera, which were dried on panels on an identity card, and if the grouping could be carried out directly on this card, the risk of interchanging in one way or another would necessarily be held at a minimum. Wagner (16) described in 1941 such a card for ABO groupings. In 1951, Scholl (14) published his experiences with Wagner's cards, but beyond this, the method does not seem to have been taken into use. This is, possibly, caused by the fact that the blood cells on such cards have a tendency to form rouleaux, which makes it difficult to distinguish negative from weak positive reactions. According to Scholl, Wagner's technique is not applicable for Rh groupings.

The cards to be described in the following sections have proved themselves practicable for ABO and Rh groupings, both in blood banks and at the bedside. The planning of these cards was begun in December 1950, and since February 1951 neither the cards nor the reagents have been altered materially.

CARD FOR SIMULTANEOUS ABO AND Rh GROUPING

The card (Fig. 1) is a piece of stiff cardboard, containing four panels in the upper half, which is covered with regenerated cellulose.*) In each panel, 60 mml. reagent is allowed to dry. Anti-A and anti-B for ABO grouping and anti-D (anti-Rh_0) for Rh grouping is found in that order in the panels. The serum in the control panel does not contain antibodies. The lower half of the card is for the name, result, etc.

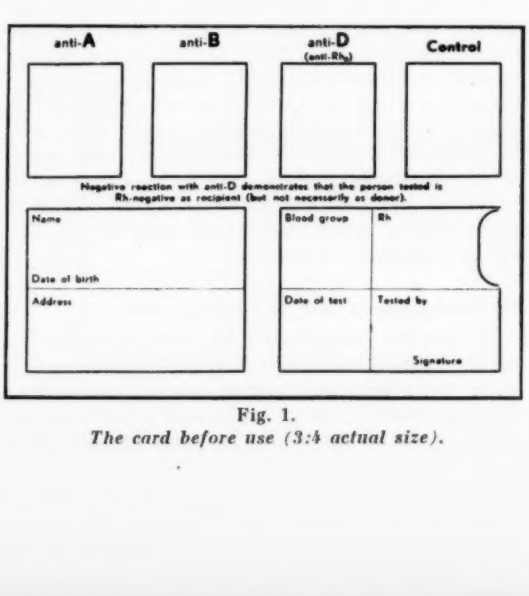

Fig. 1.
The card before use (3:4 actual size).

Molded plastic sticks and a controlled dropper pipette are delivered with the cards. The plastic sticks are to be used for measuring the capillary blood, as well as for stirring in the panels. Therefore, the sticks are fashioned with a plane terminal surface with a diameter of 3.4 mm. A hemisphere of blood deposited hereon will be approximately 10 mml. The dropper pipette should give drops of water of 45—55 mml. when held perpendicularly. It is to be used for the measuring of water and saline solution.

The blood groupings are performed directly on the cards at room temperature. The reagents are either dissolved in water, after which capillary blood is added (technique 1), or in a saline suspension of blood cells (technique 2).

Technique 1.

Technique 1 is intended for groupings of persons present. In addition to the cards, the plastic stick and the dropper pipette, the necessary equipment consists of a sterilized lancet, a watch, cotton, and clean tap water.

A drop of tap water is added to each reagent on the card with the dropper pipette, which is held perpendicular. The reagents are dissolved by stirring with the plastic stick. For each panel, the residue of liquid on the stick must be dabbed off in the panel and the stick must be thoroughly wiped. In order to ascertain that all traces of reagent are wiped off the stick it is recommended either to dip the stick in water and wipe it once more, or to make a control stirring in the control panel and then wipe the stick again. By the latter procedure a possible transfer of reagent capable of producing agglutination will be revealed, because this agglutination will appear in the control panel.

Now a large drop of capillary blood freshly expressed from the earlobe is contacted by the plane terminal surface of the plastic stick and a hemisphere of blood is removed (Fig. 2—5). The blood is mixed with the dissolved anti-A reagent on the card, after which the mixture is spread out over the whole panel. The stick is thoroughly wiped. This wiping should preferably be repeated after dipping the stick in water or making a control stirring in the control panel. The same procedure is followed in measuring blood for the other panels.

There should now be a pause of one minute in order to allow the antibodies to attach themselves

*) Regenerated cellulose is commonly known as Cellophane, which, however, is a registered trade mark for the product of special firms.

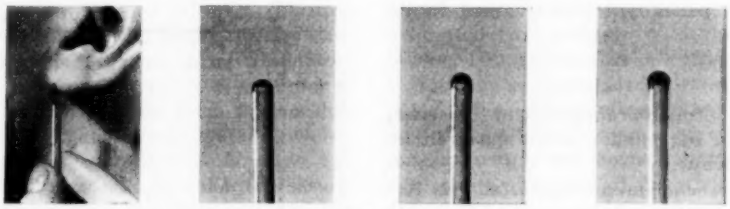

Fig. 2—5.
Measuring of blood with the plastic stick (too little — right — too much).

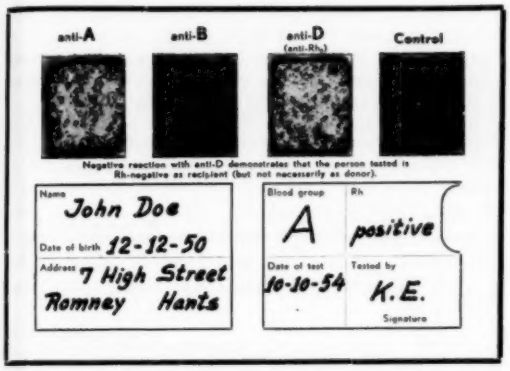

Fig. 6.
A card used with technique 1.

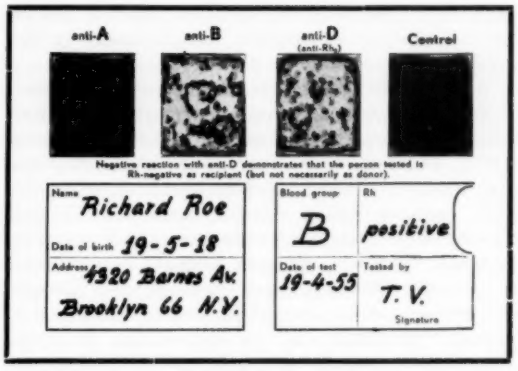

Fig. 7.
A card used with technique 2.

to the blood cells. This time should be used in filling out the card according to the information given by the patient. The card is then tilted slowly to all sides for three minutes, and is held, as far as possible, perpendicularly for 10—15 seconds at each extremity while the mixtures glide from border to border in the panels.

The result is then read (Fig. 6). After the reading, the reactions can be fixed by being allowed to dry undisturbed. In order to be certain of the homogenous appearance of the negative reactions, it is recommended to tilt the card once more two minutes after the reading. The dry cards can be protected from moisture and insects with cellulose lacquer or adhesive strips.

Technique 2.

Technique 2 is intended for investigations of blood specimens in laboratories or blood banks and particularly the blood in the pilot tubes attached to the bottles containing donor blood. The necessary equipment consists of a small test tube (e. g. 70—80 mm. × 10—12 mm.) a watch, cotton and physiologic saline solution. In addition, the card, the plastic stick and the dropper pipette. An anti-coagulant should be added to the blood sample, e. g. heparin, but not acid-citrate-dextrose solution which inhibits the agglutinations. The blood cells are sedimented by allowing the sample to stand for 6—18 hours at 4° C., in urgent cases by centrifugation. The pilot tubes should not, however, be removed from their respective bottles where, in order not to counteract the sedimentation, they should be placed vertically.

In carrying out the grouping, the plasma is drawn carefully from the specimen and the pipette is rinsed. Hereafter, 12 drops of saline solution are mixed with three drops of blood cells in the test tube, which is held in the left hand. One large drop of the mixture is placed on each panel of the card, while holding the pipette at an angle of 45°. Each panel is now stirred until all the reagent is dissolved and mixed with the suspension, which is then spread completely over the panel. The stick must be thoroughly wiped after each stirring, and it is recommended furthermore to dip the stick in water or to insert a control stirring in the control panel and then wipe the stick again. After a pause of one minute, the card is tilted back and forth as in technique 1, but only for one minute. The results are read (Fig. 7) and the card allowed to dry.

THE REAGENTS

100 ml. of reagent for all the four panels concerned are composed of 10 ml. serum, 90 ml. dextran, and two drops of 5 % heparin solution. In this article, the word dextran refers to a watery solution which in 1000 ml. contains 60 gr. dextran dry matter and 9 gr. sodium chloride. The dextran dry matter used is a mixture with a molecular weight from approximately 30,000 to 150,000. The average molecular weight is approximately 70,000. The importance of the dextran, the sodium chloride, and the heparin and of the cellulose coating

and the control panel will be explained in the ensuing sections.

The Dextran.

When used in proper proportions to the serum, the dextran is of importance to the cards in the following four ways:

1. *Dextran prevents pseudo-agglutination.* Partial drying of a mixture of serum and blood cells favours formation of rouleaux, which in several cases may resemble agglutinates (pseudo-agglutination). This can be avoided with a predominant amount of dextran (or albumin) in the solution.

2. *Dextran accelerates the processes so that heating apparatus is unnecessary.* Rh groupings, as is well-known, are normally carried out at 37° C. The following experiment showed that the Rh reaction can be accelerated by other means than heat. A strong anti-Rh serum was diluted 1:1000 with dextran and albumin (20 % albumin solution in 0,9 % saline solution), respectively. Appropriate drops of these solutions were allowed to dry on cards and examined with technique 1. Rh-positive blood was not agglutinated at 20° C. On the other hand, mixtures of the two — separately inert — dilutions could agglutinate Rh-positive blood on cards at 20° C as shown in Fig. 8. It is seen that a 1:1 mixture gives non-specific reactions with Rh-negative blood, while a mixture with nine parts of the one dilution and one part of the other gives clear and specific reactions. Other viscous solutions have shown corresponding results, although not quite so pronounced. Fig. 9 shows the results which were obtained following technique 1 with mixtures of dilutions (1:1000) of an anti-Rh serum with respectively dextran and inert serum (i. e. serum without antibodies from an AB person). The left side of the curve in Fig. 9 compares somewhat with the curve in Fig. 8, while the conditions on the right side are affected by the previously described tendency of serum to cause pseudo-agglutination. For practical usage the proportion of dextran and serum on the cards is, as stated, nine to one, which gives the necessary acceleration for Rh groupings with technique 1.

3. *Dextran activates incomplete sera.* Sera which can agglutinate Rh-positive blood cells suspended in saline are so rare, that the specialist laboratories have difficulty in obtaining a sufficiency for their own investigations. Race (11) and Wiener (17) demonstrated in 1944 the existence of incomplete Rh antibodies which attach themselves to Rh-positive blood cells without agglutinating them in saline. Sera with such Rh antibodies are available in quantity. They became practicable as test sera for Rh groupings when Diamond et all. (2, 3) in 1945 described how these sera could cause agglutination of Rh-positive cells in mediums with a sufficient concentration of plasma, serum, or albumin. In these

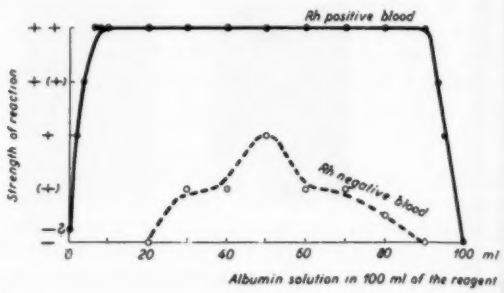

Fig. 8.

The reactions obtained by technique 1 with reagents consisting of, respectively, a 6 % solution of dextran in physiologic saline solution (left side), a 20 % solution of albumin in physiologic saline solution (right side) and mixtures of these. All reagents contained 0.1 ml. anti-Rh serum in 100 ml. reagent.

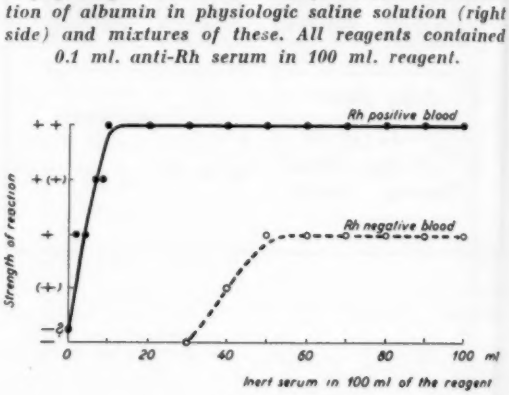

Fig. 9.

The reactions obtained by technique 1 with reagents consisting of, respectively, a 6 % solution of dextran in physiologic saline solution (left side), an inert AB serum (right side) and mixtures of these. All reagents contained 0.1 ml. anti-Rh serum in 100 ml. reagent.

mediums the incomplete antibodies were activated, according to Wiener, Hurst and Sonn-Gordon (18) by a protein complex which they called conglutinin. The viscous solutions of other high molecular materials exhibited these same activating or completing characteristics, which is why Wiener et all. named them conglutinin-substitutes. This applies to acacia (8), gelatine (4), and polyvinylpyrrolidon (5, 7, 15).

Since dextran is also a conglutinin-substitute (1, 6, 12), it is possible to use incomplete anti-Rh sera on the cards.

4. *Dextran works as a binder.* On many materials a dextran drop will adhere firmly when dried. Dried unmixed serum or albumin, on the other hand, peels off easily.

The Sodium Chloride.

According to technique 1, the salt concentration in the reaction mixture is approximately one per cent. An increase in the salt concentration to approximately two per cent increases the strength

of reaction. With still higher salt concentration (3—3.5 %) inhibition occurs. With ordinary ABO groupings, Pagniez (10) has made similar observations. The sodium chloride concentration of 2 % gives rise to still another form for acceleration, which can replace the temperature acceleration of Rh groupings on the cards. This salt acceleration is a little stronger than the serum-dextran acceleration. However, the two accelerations are so strong when combined that even reactions which should be negative become non-specific positive. If the two per cent salt acceleration is to be utilized, the serum-dextran acceleration must consequently be rejected. It so happens that neither test serum nor dextran can be dispensed with in the reagent. However, the 6 mml. serum in the reagent per panel compares somewhat to the amount of plasma in the capillary blood which is added to each panel with technique 1. This plasma can be excluded if the reagents are redissolved in a saline suspension of blood cells without any appreciable plasma admixture. This is the basis for technique 2.

The Heparin.

The heparin is added to the reagents in consideration of technique 1, where it prevents fibrin precipitation and coagulation of the capillary blood. Larger additions of bovine heparin should be avoided, since it may contain A substance.

The Cellulose Coating.

In addition to its dependency on the reagent, the purity of the negative reactions is dependent upon the surface on which the grouping is made. On paper, cardboard, parchment, various plastics, glass and gold, the reactions will be more or less non-specific positive. Certain cellulose plastics can be used, but regenerated cellulose is superior to all other materials thus far examined. With the aforementioned reagent and the proper technique, the negative reactions will appear completely homogenous on regenerated cellulose. The well-known adhesive strips of regenerated cellulose were used to coat the cards. The adhesive resin of these strips forms, in addition, a water-proof barrier from the cardboard base. It is possible that the characteristics of this cellulose film are attributable to the fact that when moistened it becomes negatively charged just as the blood cells, and thus can work as an emulsion stabiliser — in solid form — for the blood cells as they gradually settle in the reaction mixture.

The Control Panel.

The control panel, which contains no antibodies, should react negatively. By certain diseases (e. g. acquired haemolytic anaemia) the blood may contain auto-agglutinins, wherefore the cells agglutinate in all the panels. In these cases the agglutination in the control panel indicates, that also the agglutination in the other panels may be non-specific only. It can thus be prevented that patients with these diseases are erroneously grouped as AB Rh-positive.

With the recommended stirrings in the control panel after each stirring in another panel, the control panel, furthermore, serves to expose any reagent which has been transferred because of inadequate cleansing of the stick.

THE INFLUENCE OF VARIOUS CONDITIONS ON THE REACTIONS

Dry test sera will keep for many years. Dry cards have revealed unchanged reactions after (so far) two years of anhydrous storage at 20° C. and 37° C. In humid or fluid condition, however, the test sera are quickly weakened at these temperatures. This is of especial importance for the dextran-mixed reagents, because dextran is hygroscopic and in concentrated solutions can precipitate the serum proteins irreversibly. The reagents on some unpacked cards thus became completely inactive during a damp summer month.

The cards should be utilized at room temperature. At 37° C. the Rh reactions are slightly stronger, at 4° C. they are slightly weaker, but seem weaker still because the A and B reactions are fortified at this temperature. At low temperatures, non-specific reactions may, furthermore, appear in all the panels simultaneously, caused by cold agglutinins.

Increased sedimentation rate depending upon a tendency to rouleaux formation in the investigated blood has no influence on the reactions on the cards. Even with blood from a particular patient suffering from multiple myeloma, with a sedimentation rate of 176 mm/15 minutes, the negative reactions remained clear-cut negative.

Diamond and Abelson (2) state that the Rh reaction with incomplete test sera decreases with diminishing blood cell concentration. This has not the expected influence on the reactions with technique 1, because a decreased amount of blood cells in the added capillary blood is accompanied by an increased amount of plasma, and thus also by increased serum-dextran acceleration (see Fig. 9). But the haematocrit value plays a part, as the agglutinates become smaller, where there are fewer blood cells to form them. At a haemoglobin concentration of 10 % (on the Haldane scale and developed by diluting with plasma) the reactions are difficult to read. At a concentration of 20 %, the reading is not difficult in a good light. However, if the panels become noticeably pale, it is recommended to retest the blood following technique 2.

Provided that good test sera are used, it is often possible to find a remarkably strong agglutination of A_1 blood in the anti-A panel, while blood with the weak A_2 — also in A_2B — agglutinates medium strong. A_3 blood gives small

agglutinates on an unagglutinated background. Still weaker A forms (e. g., in A_3B blood) which are very rare and without clinical importance in transfusion may escape notice on the card.

After larger blood transfusions, the blood cells of the donor may dominate in the patients blood. For this reason also, the groupings must always be carried out before a transfusion is given.

POSSIBLE SOURCES OF ERROR

The influence of severe anaemia, of the very rare and weak A forms and of blood transfusions have been mentioned. It should be added, that any deviation from the directions given implies the possibility of a doubtful or erroneous result. The specifications for the liquid amounts are of special importance and must be strictly observed.

Too much water with technique 1 reduces not only the antibody concentration, but also the salt, protein and dextran concentrations. This is of the greatest importance to the Rh reaction, which is especially dependent on the accelerating and activating abilities of the protein-dextran concentrations. While the ABO reactions are only slightly weakened when each reagent is redissolved in two drops of water of 50 mml, instead of only one, the Rh reaction after this dilution will not generally appear within the reading time. The Rh reaction is already clearly weakened with 50 % excess water. It is for this reason that the drop of water should be measured with a controlled dropper pipette, which should be held exactly perpendicular with technique 1. Reduction of the concentration will also be the result if some of the reagent is not dissolved and if there is only a small cellulose surface to absorb the water because of failure to spread the mixture completely from border to border of the panel.

With the addition of too little liquid the mixtures in the panels can become too viscid to move from border to border when tilted, a fact which can easily be observed. This happens most easily to beginners, who are slow about the grouping. In these cases, the blood cells have little or no possibility for contact and agglutination and the grouping must be redone.

Due to inadequate cleansing of the plastic stick, the reagent can be transferred from one panel to the next, where it can cause an incorrect agglutination. The anti-D of the Rh panel has an especially high titer. When a control stirring is made in the control panel after each contact with the reagent in one of the other panels, any reagents which have been transferred will be exposed in the control panel.

It should be noted, that the positive reactions are weakened by too little blood and by quick and superficial tilting of the card, because such a procedure gives the blood cells too few possibilities for contact in the viscous medium. By technique 2 agglutinates, which have already been formed during the stirring can be destroyed, if the stirring continues for several minutes per panel. The homogenous appearance of the negative reactions (also in the control panel) can be disturbed

— following technique 1, by the addition of a double portion of blood or blood in the beginning stages of coagulation
— following technique 2, if the blood cell suspension contains plasma and
— after the readings, if the card does not lie horizontal and undisturbed while drying.

PRACTICAL RESULTS WITH THE CARDS

From April 1, 1952 until March 17, 1953, 6591 ABO and Rh groupings on the cards were carried out in practice. Of these, 2881 with technique 1, and 3710 with technique 2. In all these cases, however, the later recommended control stirrings in the control panel were not performed. The largest number of the investigations were carried out by laboratory assistants from the blood bank at Copenhagen's Municipal Hospital and the central laboratory at Copenhagen's County Hospital. Some investigators have made so many groupings, that they can now be considered very skilled in the use of the cards. Nonetheless, many of the investigations were carried out by doctors or laboratory assistants, who personally have carried out only one or very few groupings. All the 6591 investigations were compared with the results which were obtained by forwarding blood samples to a specialized blood grouping laboratory.

In two cases the ABO and Rh groups could not be read from the card, since there was agglutination in all the panels, including the control panel. This was caused by auto-agglutinins. Two ABO and four Rh errors were made. The one ABO error was owing to the accidental deposition of some anti-A reagent in an anti-B panel during manual preparation of the cards. On this particular card, an A person was erroneously grouped as AB. Such an error cannot happen with industrial production of the cards. The other ABO error occurred when the very weak and clinically insignificant A factor in an A_3B person was missed by technique 2. The six A_3 persons included in this material, were all correctly determined, just as were all A_2 and A_2B persons. The Rh errors consisted of grouping as Rh-negative four Rh-positive patients. In five other cases, the reactions were doubtful, although they should have been positive. These nine investigations were carried out by beginners with their first, second or third use of technique 1, and the errors were caused either by the addition of too much water or by partial dissolution of the anti-D reagent.

The blood groupings at the specialized laboratory comprised an ordinary ABO grouping of the blood cells supplemented by back-typing, and

two separate Rh groupings. It was not settled in how many cases retesting was performed because of discrepancies in these results. The errors which appeared in the grouping results from the laboratory were caused by the mixing-up of blood samples from an O Rh-negative, an A Rh-positive and an AB Rh-negative person with three samples from O Rh-positive persons. It is assumed that this occurred at the time of venipuncture.

Approximately 4800 of the ABO results were compared with an ordinary ABO grouping on slides carried out on the spot. This control revealed no errors in the grouping by cards, since the previously related A_3B was also determined as B by the slide method. On the other hand, a B person was erroneously recorded as AB by the slide method.

Since March 17, 1953, approximately 30,000 cards have been used without control determinations. In approximately 15,000 cases the ABO grouping was, however, supplemented with back-typing, which in no case revealed any errors on the cards. Amongst the remaining investigations, six errors were reported. Two of these appeared when (as mentioned earlier) the reagents on some cards were destroyed by moisture in the damp summer of 1953. This was before moisture-proof packings were introduced. A single investigator made two investigations with technique 2, during which an O Rh-positive and an A Rh-positive person were both grouped as O Rh-negative. The investigator had, on this occasion, stirred from five to seven minutes in each panel. It was proved that the agglutinates can be destroyed by such exaggerated stirring. Finally, two beginners, as a result of inadequate cleansing of the plastic stick, transferred reagent recently, from the anti-D panel to the anti-B panel. In this case, two O Rh-positive people were determined as B Rh-positive.

ADDITIONAL CDE GROUPINGS ON CARDS

It has gradually become well-known that anyone who lacks the Rh factor D is Rh-negative as a recipient of blood, while only those who lack the Rh factors C and E as well are also Rh-negative as donors. During the past half year the blood banks at Copenhagen's Municipal Hospital and Copenhagen's County Hospital used special cards for the additional CDE grouping of those donors who were classified Rh-negative as recipients in the primary investigations with anti-D. The CDE cards were used in the same way as the cards for ABO and Rh groupings. However, in consideration of the weaker anti-E reagent, the result was read two minutes later during an extra tilting of the card. Among 1179 donors who were Rh-negative as recipients, 110 were found to be Rh-positive as donors (64 because of the C factor, and 46 because of the E factor).

DISCUSSION

Blood groupings with dried reagents, which are redissolved at the time of grouping, must necessarily be based on exact execution. With simultaneous ABO- and Rh groupings on the cards, the demands for accuracy comprise the ensuing points:

1. One drop of liquid must be placed in each panel with the dropper pipette in the correct position (perpendicular with technique 1 and inclined at an angle of 45° with technique 2).
2. All the reagents must be dissolved and the mixture of blood and reagent must be distributed so that each panel is completely covered.
3. The stirring stick must be carefully cleansed after each stirring.

It has been proved that these indispensable regulations, which should be stressed for beginners, have in practice been adhered to by the investigators who have carried out more than three groupings.

In return for the demands for accuracy, the cards make possible a rapid and reliable ABO and Rh grouping, which is simple to learn and easy to carry out at the bedside or in the laboratory. The method excludes the possibility of errors as a result of auto-agglutinins, increased sedimentation rate and interchanging of test sera. For smaller hospitals, it can be an important factor that the investigation does not require any specialized apparatus, and that the test serum reagents on the cards are valid for two years at room temperature. For larger hospitals, it is of especial importance, that interchanging of the specimens from the patients can be considered practically impossible with technique 1; and that the cards (at any time after the determination) allow control of the correctness of the readings and evaluation of the precision with which the determination is carried out. Technique 2 will give the blood banks a corresponding security against interchanging of the donor specimens as long as the pilot tubes are not removed from their respective bottles.

When desired, the ABO grouping on cards can, as any other ABO testing of blood cells, be supplemented with an investigation of the serum against known A and B cells. It is not necessary to carry out this back-typing at the same time and place as the blood cell investigation. It should be emphasized that the main value of back-typing is the exclusion of some possible sources of error which are, however, without influence on grouping by card.

SUMMARY

A new and simple method for simultaneous ABO and Rh grouping without specialized apparatus is presented. The testing is carried out at room temperature on a card which can be pre-

served after the determination. The use and preparation of the cards, the basic serologic conditions and the possible sources of error when using the cards are described in detail. An account is given of the experiences gained from 6591 controlled determinations and about 30,000 determinations without regular controls. It is emphasized that the instructions given must be followed exactly, especially the directions for measuring of liquids and cleansing of the stirring stick. Beginners should be supervized. The advantages of the method for both large and small hospitals are mentioned.

Address: Nordisk Insulinlaboratorium, Gentofte, Denmark.

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References:

1) *Bonnel, P. H.:* Rev. hémat. 1951, *6:* 95.
2) *Diamond, L. K. & N. M. Abelson:* J. Lab. & Clin. Med. 1945, *30:* 204.
3) *Diamond, L. K. & R. L. Denton:* J. Lab. & Clin. Med. 1945, *31:* 821.
4) *Fisk, R. T. & C. A. Mac Gee:* Am. J. Clin. Path. 1947, *17:* 737.
5) *Formaggio, T. G.:* Il Sangue 1950, *23:* 243.
6) *Grubb, R.:* J. Clin. Path. 1949, *2:* 223.
7) *Hummel, K.:* Ztschr. Immunitätsforsch. 1950, *107:* 418.
8) *Levine, P.:* Am. J. Clin. Path. 1946, *16:* 597.
9) *Moreau, P.:* Monde méd., Paris 1939, *49:* 854.
10) *Pagniez, N. F. M.:* Rapp-s Commun-s 4. Congrès internat. Soc. internat. Transfus. sanguine 1951. Paris 1952, p. 449.
11) *Race, R. R.:* Nature, London 1944, *153:* 771.
12) *Richardson Jones, A.:* Nature, London 1950, *165:* 118.
13) *Salber, R.:* Über Fehlerquellen bei Massenblutgruppenbestimmungen. Uster 1951, p. 41.
14) *Scholl, F.:* Wien. klin. Wchnschr. 1951, *63:* 212.
15) *Spielmann, W.:* Ztschr. Immunitätsforsch. 1950, *107:* 503.
16) *Wagner, G. F.:* Dtsch. Mil. Az. 1941, *6:* 164.
17) *Wiener, A. S.:* Proc. Soc. Exp. Biol. 1944, *56:* 173.
18) *Wiener, A. S., J. G. Hurst & E. B. Sonn-Gordon:* J. exp. Med. 1947, *86:* 267.
19) *Wiener, A. S., D. N. W. Grant, L. J. Unger & W. G. Woorkman:* J.A.M.A. 1953, *151:* 1435.

BLOOD GROUPING IN A BLOOD BANK BY THE ELDON METHOD

By KELL JORDAL

Until the requirements of Rh determination became apparent, blood grouping prior to a transfusion did not present any great problem for physicians in Danish hospitals. On account of the well standardized anti-A and anti-B test sera, prepared by The Danish State Serum Institute (Statens Seruminstitut), ABO grouping could, as a rule, be performed easily and rapidly and the method might be considered accurate.

Until 1952, all Rh grouping was performed in the State Serum Institute. This centralization of Rh typing certainly implied great reliability in typing but implied simultaneously that the hospitals had to wait for the result of blood typing. Even with measures such as emergency determinations with express delivery of blood samples and reply by telegram, the arrangement was not satisfactory in practice. Situations occur daily in a hospital in which rapid blood typing is essential. If the accepted principles for blood transfusion should be followed, the result would ensue that in a disproportionately large number of cases Rh negative blood would have to be administered to recipients whose Rh type was unknown. With the great increase in the number of blood transfusions the difficult situation therefore frequently arose that Rh negative donors were not available to a sufficient extent.

From Surgical Department 1, The Municipal Hospital (Kommunehospitalet), Copenhagen. Head: Professor *Otto Mikkelsen.*

Serological experts considered Rh typing so difficult and uncertain in the hands of untrained personnel that it was regarded inadvisable to introduce measures for decentralization of Rh grouping.

A solution, however, presented itself when Eldon, working in the State Serum Institute, in 1950 demonstrated that convenient and reliable ABO and Rh grouping could be undertaken employing his card system.

A more detailed review of Eldon's method falls outside the scope of this article. The reader is referred to Eldon's own publication on the subject. The object of the present article is to show that a blood bank in a large hospital may be conducted employing Eldon's method and to demonstrate the advantages offered by this method compared with methods previously employed.

As already mentioned, the method was suggested by Eldon in December 1950. The method has been tried out in practice since March 1951, when it was first employed in the blood bank of the Municipal Hospital, Copenhagen.

The Eldon Card (see p. 34) upon which the blood typing is performed, is a rectangular piece of pasteboard, the upper half of which is covered with a cellophane film. Upon this film, specially prepared test serum reagents are present in a dry state, as in each of the four panels a drop of serum has been allowed to dry. The type of the


anti-A
anti-B
anti-D (anti-Rh)
Control
Negative reaction with anti-D demonstrates that the person tested is Rh-negative as recipient (but not necessarily as donor).
Name Ann Marshall
Date of birth 31-5-15
Address Champs fleurs Port of Spain
Blood group A
Rh positive
Date of test 11-9-54
Tested by B.P.
Signature
Name Peter Allen
Date of birth 2-11-53
Address 477 Castle Str. Edinburgh 2
Blood group A
Rh negative
Date of test 5-9-54
Tested by E.H.
Signature
Name Robert Hall
Date of birth 17-12-38
Address 1 St. Andrew Crescent. Leeds 5.
Blood group B
Rh positive
Date of test 13-9-54
Tested by U.H.
Signature
Name Charles Taylor
Date of birth 15-1-06
Address 296 Chichester Street. Belfast
Blood group B
Rh negative
Date of test 8-8-52
Tested by K.E.
Signature
Name George Nicholson
Date of birth 18-7-22
Address 17 West 71 Street N.Y. 23
Blood group O
Rh positive
Date of test 4-1-55
Tested by O.R.
Signature
Name Christopher Smith
Date of birth 2-4-32
Address 32 The Drive Sussex
Blood group O
Rh negative
Date of test 17-5-54
Tested by U.H.
Signature
Name Mary Blackstone
Date of birth 16-6-88
Address 57 Sloane Square London S.W.1.
Blood group AB
Rh positive
Date of test 19-2-53
Tested by K.M.
Signature
Name Henry Smith
Date of birth 18-11-24
Address 15 Kings Walk Chancery Lane E.C.4.
Blood group AB
Rh negative
Date of test 1-6-54
Tested by A.M.
Signature

Fig. 2.

serum is indicated above each separate area so that errors are excluded. The lower half of the Card is reserved for identification of the individual whose blood is to be typed and the result of the typing is also recorded on this section of the Card.

According to Eldon's original instructions, typing is performed by dissolving the dried test sera on the card in pure water, after which a certain amount of capillary blood is added to each drop of serum. After stirring and rocking of the card, the reactions may be read directly at room temperature after the lapse of 3 minutes. By this procedure, typing must be performed at the bedside of the individual whose blood is to be grouped. This implies the great advantage that errors of identity are excluded, but simultaneously it makes the method slightly more cumblesome when several cases for typing are concerned, widely dispersed within a hospital.

For particular blood bank purposes we have, at the Municipal Hospital, in co-operation with Eldon employed a slightly different method which has rendered just as good and accurate results. At the Municipal Hospital in Copenhagen, all blood typing is centralized in a blood bank. The blood samples are withdrawn in the various departments in Wassermann tubes already containing a drop of heparin. The stabilized blood sample is delivered to the blood bank where the typing may be performed immediately in an emergency or when convenient during the daily routine.

After centrifuging of the blood sample, the plasma is removed by a pipette. Approximately 1 ml. blood cells are placed in a small test-tube (approximately 75 × 10 mm.). The test-tube is filled up with physiological saline and thereafter centrifuged at high speed for 3 minutes. The saline is again removed by pipette and the test-tube once more filled with saline. After meticulous shaking of the centrifuged blood cells, the suspension is ready for grouping.

With a standard dropper pipette, a drop of the suspension is placed on each of the 4 areas on the card, care being taken to hold the pipette at an angle of 45° to the underlying card. By means of a plastic rod which is slightly flattened at one end, each area is carefully stirred so that the dried reagents are entirely dissolved. Stirring is commenced in the control panel furthest to the right and is continued thereafter in the other areas from right to left. The rod must be dipped in water and carefully dried with cottonwool or wood wool on changing from one area to another. This drying must be very thorough so that no serum from the one area is transferred to the next. When stirring has been completed in the course of about one minute, the card should be laid aside for a minute which may be utilized to enter the name of the individual concerned and the other information in the appropriate spaces. The card must now be rocked for one minute, both backwards and forwards and from side to side. The reactions become apparent by agglutination in the areas and the card may be read off immediately according to the headings printed above the areas. The reactions appear from Figure 2.

During the 2-year period from March 1951 till March 1953, all blood typings performed on Eldon Cards in the Municipal Hospital were controlled by the sending of control blood samples to the State Serum Institute. No special account of errors in typing from the blood bank in the Municipal Hospital is available but out of 6,591 blood typings on Eldon Cards, 2 incorrect ABO determinations were disclosed and 4 incorrect Rhesus determinations. These determinations were carried out in various hospitals in Copenhagen and in some cases by inexperienced personnel. One of the ABO errors was caused by a technical error in the preparation of the card, a source of error which now has been eliminated. The other ABO error was due to failing reaction for the weak A-factor in A_3B. The usual control typing determination on a slide did not, however, show any A-factor either. In the material, a total of 6 A_3 individuals are present all of whom were correctly typed on the card. The errors in Rh determination were that 4 Rh positive individuals were typed as Rh negative. These typings were all carried out by beginners who had not received sufficient instruction and consequently had not adhered to the prescribed instructions for the performance of the typing. In summing up it may be said that the Eldon Cards have rendered reliable blood typing determinations as long as the rules stated for their employment were adhered to.

Since April 1953, all blood type determinations in the blood bank of the Municipal Hospital were undertaken exclusively by the Eldon Card method. As is usual in all blood banks, simultaneous controls from the serum of the blood samples were undertaken with blood cells of known type (back-typing). From April 1953 to March 1955 the total number of type determinations was 9,538 of which 6,754 concerned hospitalized patients while 2,784 were determinations on donors for these patients.

The patient material comprizes patients with all types of diseases, both adults and children. The material is thus unselected. The Municipal Hospital disposes over approximately 1,000 beds. The number of transfusions per annum since the blood bank was instituted appears from Figure 3.

Annual Number of Blood Transfusions Since the Establishment of the Blood Bank.

1948	1,182	Transfusions
1949	1,283	—
1950	2,562	—
1951	3,970	—
1952	4,942	—
1953	5,688	—
1954	6,603	—

Figure 3.

The Distribution of the Blood Groups Determined During the Period April 1953—March 1955 Exclusively by the Eldon Card Method.

	Rh positive	Rh negative	Total	%	Normal Percentage in the Danish Population
A	3527	624	4151	43,5	44
O	3418	592	4010	42,0	42
B	790	142	932	9,8	10
AB ...	354	91	445	4,7	4
	8089	1449	9538		

Rh positive ..... 8089 84,8 % (Normal 85 %).
Rh negative ..... 1449 15,2 % (Normal 15 %).

Figure 4.

The incidence of the various blood groups among the groupings undertaken appears from Figure 4. For comparison, the average distribution of the various blood groups in the Danish population is also given.

THE ADVANTAGES OF THE ELDON CARD METHOD

In our experience, the employment of the Eldon Card method for blood typing in a blood bank has offered great advantages.

Among the advantages it should be primarily stressed that the Eldon Card method is accurate both for ABO and for Rh typing. If the instructions are followed, clear and unmistakable reactions are obtained which may be read without difficulty. The possibility of mixing up sera is excluded as the types of the various sera are printed on the card above the areas in which the sera are present in a dry state. The control area on the card is a safeguard against pan-agglutination, a source of error which, as a rule, cannot be revealed by the usual methods of typing (Figure 5).

The Eldon Card method is simple and readily grasped. After brief instruction, the typing may be entrusted to any physician or laboratory assistant. The card method renders control of the reaction possible as the final reaction may be checked at any time. As the investigator signs the card it is always possible to trace the individual who has carried out the typing. Any error which occurs is thus traceable.

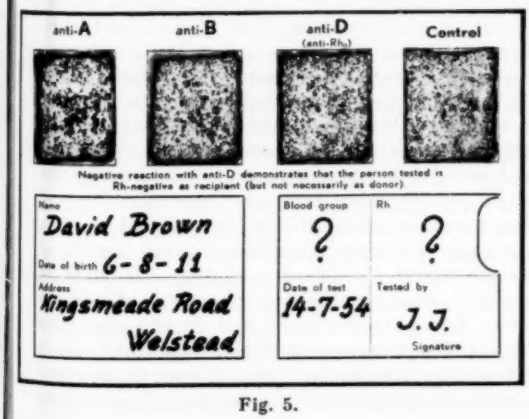

Fig. 5.

As an example of the fact that the Eldon Card method can safely be entrusted to various physicians after brief instruction, it may be mentioned that during the period April 1953—April 1954, 537 emergency blood typings were carried out in the blood bank. 296 out of these were carried out by various house surgeons from the surgical departments at hours when the permanent staff of the blood bank was off duty.

Blood typing employing Eldon Cards is rapid. In emergency cases, a reliable ABO and Rh determination may be available in about 3 minutes. The typing may be carried out anywhere with simple equipment. The significance of this scarcely needs any further explanation. The importance of administration of type-specific blood to the patients has been established and also the risk of administration of incompatible blood. Another aspect of the problem should, however, be borne in mind: on account of the rapid and convenient possibility of Rh determination it has proved possible to limit the requisitioning of Rh negative blood to an order which corresponds more or less to that anticipated from the incidence of Rhesus-negative individuals in the Danish population (Figure 6).

Percentage Utilization of Blood in the Various Blood Groups.

	Rh positive	Rh negative	Total	Normal Percentage in the Danish Population
A	28,0 %	10,7 %	38,7 %	44
O	38,9 %	8,8 %	47,7 %	42
B	7,4 %	2,5 %	9,9 %	10
AB	3,1 %	0,6 %	3,7 %	4

Rh positive ..... 77,4 % (Normal 85 %).
Rh negative ..... 22,6 % (Normal 15 %).

Figure 6.

By employing the Eldon Card method, the requirements of laboratory equipment in a blood bank become very modest. The outfit found in any hospital laboratory suffices: centrifuge, rinsing bowls, test-tubes, stirring rods and physiological saline.

The typing takes place at room temperature. Ordinary changes in temperature do not affect the reactions.

The Eldon Cards can be filed at room temperature and at this temperature they will keep for a long period. Thus no fading in the reactions was demonstrable in cards which had been filed for 2 years at room temperature. This latter condition is of particular significance in hospitals where relatively few typings are carried out. By filing Eldon Cards, the possibility of reliable

typing is ascertained, independantly of test sera which may become inactive on account of age or by unsuitable storage conditions.

As a consequence of the simplicity of the Eldon Card method, the requirements of personnel to carry out the blood typing are also modest. Frequently, it will be possible to employ the ordinary staff of physicians and laboratory assistants. The work and the system of safety precautions may be simplified in a blood bank employing Eldon Cards.

SUMMARY

The Eldon Card method for blood type determinations (ABO and Rh) was tried out in the blood bank of the Municipal Hospital, Copenhagen, since March 1951. The method has proved very reliable. It is rapid and convenient and enables any hospital to carry out reliable ABO and Rh determinations in the course of 3 minutes, employing the ordinary hospital equipment and personnel. The rapid Rh determination has limited the requirements of Rh negative donors. The demands for Rh negative blood correspond to the quantities anticipated from the incidence of Rh negative individuals in the population.

It is a pleasure to thank Mrs. Ulla Hartmann, whose skill and accuracy in blood grouping and generous cooperation made this study possible.

THE PREVENTION OF HAEMOLYTIC TRANSFUSION REACTIONS

By P. H. ANDRESEN

The steps to be taken to prevent haemolytic reactions can be described in a few words: thorough organization of the work in the blood bank.

In Denmark the organization of the transfusion service is only in its infancy, and until recently we had only one blood bank, which was an independent institution with a serological specialist as a chief. In the future we will get a few independent, regional blood banks, each with a serological specialist as a chief, and in addition large and small subcentres at each hospital without serological specialists, but in close contact with the nearest regional blood bank.

However the blood transfusion service is organized, one will always meet the same problem: part of the work in connection with blood transfusions will be done by doctors or assistants without a serological education, most often without any education in laboratory-work and usually with very little understanding of the problems. This is not a theoretic assertion, but the result of the study of the published papers on dangerous transfusion-reactions. These papers show very clearly that most of the reactions are due to complete ignorance of personnel concerned and lack of control and organization. Therefore, when we plan the work to decrease the risk of haemolytic reactions, we must realize that the blood banks alone are responsible for the serological examinations and guidance. The hospital departments' responsibility should only be carrying out very simple examinations and practical organization in strict accordance with the requirements of the blood bank.

In the first place we must realize, what demands the blood bank must make on itself.

From the Blood Bank, Bispebjerg Hospital, Copenhagen. Head: *P. H. Andresen.*

The first condition is that the blood bank can absolutely guarantee that the blood it delivers, or the donors it provides, belong to the blood-group stated on the label or on the donor's certificate. This is attained by means of a well organized, controlled registration of donors, blood bottles and blood samples, and further by an extremely safe blood-grouping.

At the new-erected blood banks the question arises how to fulfill the primary condition of the bank: That it can absolutely guarentee that the blood it delivers or the donors it provides belong to the blood-group stated on the label or on the donor's certificate.

At the Bispebjerg Hospital, Copenhagen, the following system is in use:

At the donor's arrival four different forms made up as a small booklet are filled in with the donor's name, birthday, address, etc. The first page, on which the donor certifies that he has never suffered from syphilis, malaria or jaundice, is kept in the blood bank and decides the fate of the blood. The next page is a personally signed letter of thanks for the donation, which proves that the donor has really been in the blood bank. The third page, a yellow card, which is the most important part of the system, is immediately after the bleeding tied to the bottle. The fourth page is a form for the Wassermann-Kahn-Meinicke test. All four pages bear the same number and letter, and six printed numbers corresponding to the number on the card complete the system. Four of the numbers are put on four blood-samples, obtained just after the bleeding. One sample of clotted blood for blood grouping, one glass of citrated blood for keeping, one glass of citrated blood for cross-matching, and finally one glass for the Wassermann-test. The fifth number, which follows the card, is torn off and used in the case-report of the recipient in order to indicate which

blood that is used for the transfusion. The sixth number is fixed to the card and will be a permanent guarantee for the corresponding between the number and the card.

The application of printed numbers is effected in all blood bank examinations to prevent mislabelling, the most dangerous and frequent mistake in a blood bank.

When the donor's blood group is known, we paste a ticket with the blood group, covering the donor's name on the yellow card, and now the bottle is anonymous. The tickets have the Scandinavian standard colours, and the ticket on each bottle will be controlled and signed before it leaves the blood bank.

In the middle of the card, the name of the recipient is stated, and the inspecting doctor signs that a cross-matching has been made. Finally the doctor that carries out the transfusion must sign the card and state, whether the transfusion has given any reaction, and at last the card is returned to the blood bank (about 95 % are returned).

By means of this card and the red front page in the booklet the blood bank will later be able to give all information about the transfusion.

Having thus protected the blood bank against mistakes, the most important work will be an absolutely safe blood grouping by the ABO-system. In order to secure the good result of the examination, two assistants always work together, so that all results are seen at least by four eyes, and there will immediately be read proofs at the entered results. In this way the blood bank can guarantee for the blood, which is delivered.

Now is the question: how to provide donors with the same guarantee. It must be a definite demand that donor certificates are only issued to persons, on whom blood grouping has been made on two different occasions.

A hospital section without its own blood bank should, as far as possible, only use donors, which are guaranteed from a blood bank or an approved special-laboratory. Blood from relatives or new voluntary donors, which the hospitals have procured themselves, should not be used until the blood grouping is available from a special-laboratory. Moreover it is hereby attained that the blood has remained so long time under refrigeration that the risk of transmission of syphilis has disappeared.

The first task of the blood bank is to make security as regards the donor's and the patient's blood-groups after the ABO-system. The next task of the blood bank is to prevent the recipient from producing other blood group antibodies — and in connection with this to prevent blood transfusion reactions, in case such antibodies are already made.

As the Rhesus system is far the most important factor here, this is to be mentioned at first. Of all the antigens that may be in question, the factor D is so dominating that it must be considered first. The most important demand is that all Rhesus-negative persons, that is persons, who are D-negative, must have D-negative blood, and all donors must therefore be examined very carefully for the receptor D, and all donors, who do not give agglutination reaction with anti-D, must therefore be further examined with incomplete anti-D in connection with Coomb's indirect test.

As also the factors C and E in some cases will produce antibodies, it must further be demanded that all donors, who prove to be D-negative, also must be examined regarding the receptors C and E, in such a way that a donor called rhesus-negative is C-negative, D-negative and E-negative.

Of course one will not in this way be quite sure that the recipient will not produce any antibodies. Perhaps there is a special reason to pay attention by all donors to the Kell-factor, but as this will involve some practical difficulties, the question is temporarily postponed. Also by examination of the patients' blood, the blood bank can do its share to prevent the haemolytic reactions. By examinations after the ABO-system all the same requirements must be considered as by the examination of the donor's blood, but besides one must put stress on the examination of all the irregular agglutinines one meets, also those only reacting by room-temperature.

By examination for the Rhesus-system it is again the D-factor that must be considered. The use of Coomb's indirect test in all cases is not required here, but in return the serum of all D-negative ought to be examined for the presence of Rhesus-antibodies.

In many cases it is most practical that the blood bank makes the cross-matching, but if the blood bank carries out the examinations mentioned here, and if a secure organization of order and distribution is planned, it is possible fairly safely to leave the cross-matching to the hospital-sections in question. It is of great importance that this now is made by the simplest methods, which do not involve any greater possibility of mistakes and which do not require any further knowledge. On planning the cross-matching it must never be forgotten that the most important task of this is, at the last moment, to discover glaring blunders made concerning the ABO-system. When we in the blood bank also generally are content with the simple double cross-matching, this is due to the fact that incompatibility will be so rare under the conditions given here that the attention of the staff will be weakened, when for instance a complicated method is used, which maybe does not give a positive result once a year.

The demands, which must be put to the departments that undertake the blood-transfusions must be: firstly there must be an organization, which prevents mislabelling of the samples sent to the blood bank. These mistakes has been the cause of death in many instances, and in these cases the

person, who makes the mistake, alone has the responsibility.

Secondly, in each case there must be a written order, though the blood only is to be fetched from a depot attached to the department or has to be taken from a donor called in.

Thirdly, the blood must be of the group stated on the order, and this must be controlled by another person than the one, who takes out the blood from the depot or bleeds the donor.

Finally the person that gives the transfusion must ascertain that the patient's blood group is correctly stated.

The next demand is as mentioned that a clear case-report is available, concerning previous blood-transfusions and especially blood-transfusion complications, and besides for women, whether they have born children suffering from erythroblastosis. If the case-report gives anxiety about occurrence of haemolytic complications, the examination for incompatibility ought to be made in the blood bank, which now has time and knowledge to help, when it is necessary. It must be added that assistance from the blood bank is often requested on transfusion of patients with blood-diseases.

Finally I want to emphasize that we in the blood bank led by me, in the three years it has acted, have followed these principles and hereby have had only three mild haemolytic reactions, of which the two cases were due to direct mistakes. Only the last one could have been prevented if Coomb's indirect test had been made.

Besides these haemolytic reactions we have only been told about complications to blood-transfusions in about 1,8 %. In all these cases, Coomb's indirect test has been made after the transfusion between the recipient's serum and the donor's blood-cells, but apart from the case mentioned, we have not been able to detect any antibodies, which was able to give an explanation of the reaction.

In most of these cases it has been a question of evident allergic reactions, but undoubtedly there are still many sources to blood transfusion reactions, of which we do not know.

THE EPIDEMIC OF POLIOMYELITIS IN GREENLAND 1953

By BODIL ESKESEN and BODIL GLAHN

It is notable that sporadic cases of polio in Greenland are never recorded, especially when one takes into consideration the hygienic conditions and the apparently abundant possibilities for contagion. The same observation has been made of the Eskimoes in Canada, where the first epidemic broke out as late as in 1948—49 and has been described by A. F. W. Peart (1).

In »Meddelelser om Grønland« (2), A. Bertelsen gives an excellent account of earlier polio epidemics in Greenland. As early as in 1858, in the medical report from Julianehaab by L. Prosch (3), an epidemic was described which, to all appearances, must have been polio. After that there were epidemics reported in Ivigtut in September, 1913, in Sukkertoppen in July and August, 1914, in Egedesminde and Jakobshavn in the summer of 1920, in Angmagssalik from September to November, 1925, and in Sukkertoppen, Holsteinsborg, Godthaab and Egedesminde in the period from June, 1932 to November, 1933.

The epidemics in Sukkertoppen and Holsteinsborg in 1932 were described in detail by K. Hrolv and H. V. Christensen (3). The epidemic in Sukkertoppen began the 16th of June and lasted nine weeks. A total of 55 cases that were clinically manifest occurred, with 13 deaths amongst the 1538 inhabitants of the district (a disease rate of 36 per thousand). In Holsteinsborg, the epidemic began the 19th of June and lasted six weeks, and a total of 28 cases that were clinically manifest occurred with 7 deaths (a disease rate af 28 per thousand). The oldest patient were born in 1915, so there were none stricken who lived during the epidemics of 1914. In each individual small village, the epidemic rarely lasted more than eight days. Aside from a mild epidemic in Nanortalik in 1945, there had not been any further polio epidemics in Greenland until 1952—53.

In November, 1952, in connection with the serious polio epidemic in Denmark, polio broke out in the Godthaab district. The epidemic began the 22th of November and lasted three weeks. It was comparatively mild with 9 paralytic cases of whom 2 died, besides an unknown number of non-paralytic cases.

On the 8th of December, polio occurred in Sukkertoppen. Here, also, the epidemic developed comparatively mildly with 8 paralytic cases of whom 2 died, apart from an unknown number of non-paralytic cases.

The epidemics in these two districts are described in an unprinted report written by Mogens Fog-Poulsen and Bent Møller (3).

In January, 1953, a polio epidemic broke out in Umanak, which, at that time, had not had any connection with other districts for almost two months. There were 22 paralytic cases of which one died.

From the Greenland Department, Copenhagen.
(Medical consultants: *T. Thune Andersen* and *Sylv. M. Saxtorph*).

On the 14th of May, the first cases of polio occurred in Diskobugten in the Egedesminde district, where the epidemic terminated after a duration of two and a half months. In the month of August, it spread further throughout the bay area to the districts of Jakobshavn, Kutdligssat and Godhavn and finnally quieted down after a good three months' run. The last case occurred in the last week of November when the winter had long since set in with heavy frost.

Finally, in August of 1953, a minor and short-lived epidemic appeared in the district of Julianehaab with but 12 paralytic, though very severe cases, of whom 4 died.

The polio epidemic in Greenland of 1952—53 extended over a period of 12 months. Also in the past, about a year elapsed before a polio epidemic died down after having stricken a varying number of colonies. The epidemic was the most widespread so far, since it struck the inhabitants of eight of West Greenland's eleven medical districts. It reached its peak in the Egedesminde district, in regard to both the number stricken and the degree of severity of the paralyses.

An account follows of the polio epidemics in the four medical districts around Diskobugten — Egedesminde, Jakobshavn, Kutdligssat and Godhavn — which have a total of 6609 inhabitants.

Diskobugten is on the west coast of Greenland, north of the Arctic Circle, at 68° latitude, corresponding to the northernmost part of Norway. As a result of the ice conditions, these areas are cut off from any connection with the southern part of Greenland from December to May.

The population lives in one-family houses, frequently small and overcrowded. The sanitary conditions are extremely poor. Drinking water is obtained from inland lakes and is usually unclean. It must often be carried for long distances in pails, and only in the towns, during the three summer months, it is carried through pipes over the rocks out to houses or taps. In these northernly areas, the waste is taken care of by the dogs who eat everything, including excrements.

The reader is referred to Dr. Fog-Poulsen's recent account of the set-up of the sanitary system and working conditions in Greenland (4). It can be briefly stated here that in Egedesminde there is an old hospital with 40 beds, in Jakobshavn a good hospital with 32 beds, and in Kutdligssat a smaller one with 19 beds, while Godhavn has only a small infirmary with 6 beds. The first two districts named are each taken care of by two doctors while the other two each have one. Besides the nurses who have been trained in Denmark and who are connected with the hospital, there are a number of midwives in the districts, the majority of whom have been trained in Greenland and who live in the smaller villages where they also act as nurses.

The Egedesminde medical district is the next largest in Greenland with 2870 inhabitants. There are 1200 in the town itself and the rest live in 13 small villages with between 40 and 250 inhabitants in each place. The distances between these settlements are considerable. It takes over two days for the physician's boat to get to all the places in the district. The medical district of Jakobshavn lies easternmost in the bay with 1729 inhabitants, while the other two medical districts are situated on the island Disko: Kutdligssat with a population of 1343 and Godhavn with a population of 669. Also here, many are included who live away in smaller villages.

The Course of the Epidemic.

The epidemic in the Egedesminde district began on the 14th of May, 1953, in Kangatsiak, one of the southern villages, and in the course of the first three days, four patients died with symptoms of bulbar or respiratory paralysis. All four were from the village of Kangatsiak.

The further course of the epidemic appears from Fig. 1, which gives the total number of paralytic and fatal cases per week.

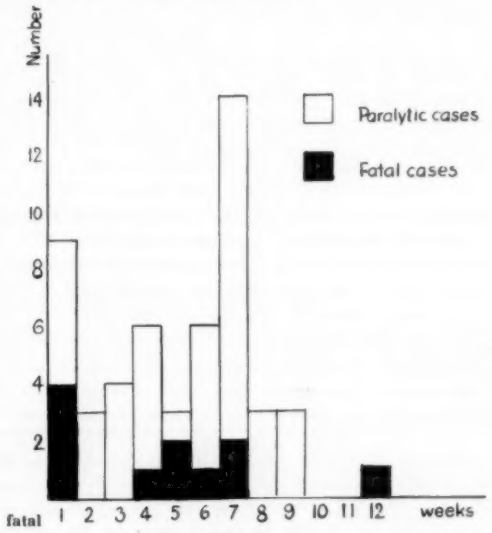

Fig. 1.
The course of the epidemic as indicated by the number of paralytic and fatal cases per week. (In Egedesminde).

A total of 75 polio patients were admitted to the hospital in Egedesminde in the period from May 4th to August 3rd, 1953. Of these, 67 had pleocytosis in the spinal fluid, and the other seven were admitted because of paralysis found during later examinations in the district. Out of the 17 villages in the Egedesminde district only 3 escaped polio. The epidemics in the various villages rarely lasted more than one or two weeks, but in the town of Egedesminde itself, the cases were scattered evenly over the length of the epidemic, which lastet more than two months.

In one particular village, Nivak, the epidemic developed almost explosively. Nivak is a little village with 128 inhabitants, and the houses are small and overcrowded. From this village, a total of 14 patients were admitted to the hospital within the course of four days: 9 paralytic of whom 2 died, and 5 non-paralytic cases. 9 were under 15 years of age, and of these, 8 had paralysis. Only one of the 5 over 15 years of age had paralysis and then only very slightly in one upper extremity. The oldest patient was 21.

As already mentioned, the population in Egedesminde is approximately 2,800 and the incidence of the disease, calculated from the 75 polio patients with pleocytosis, is 26 per thousand. But during later check-ups, a total of 45 persons were examined who had all been ill at the same time as those who had been admitted to the hospital and who presented the same pathological picture: headache, pains and stiffness in the neck, fever and possibly nausea with vomiting. The probable incidence of the disease, then, is 42 per thousand, a high disease rate heretofore unheard of.

The epidemic in the Egedesminde district took an exceptionally severe course. Of the 75 patients, 32 male and 43 female, 52 had paralysis, that is, 18 per thousand of the population (see Table 1); of these 11 died, that is 22 %, with symptoms of respiratory or bulbar paralysis, and there were no deaths in the district where there was an indication that the cause was polio.

Table 1.
Paralytic cases in Diskobugten's four medical districts compared with the number of inhabitants.

	Inhabitants	Paralytic cases	Frequency of paralysis	Deaths
Egedesminde ..	2870	52	18 per thousand	11
Jakobshavn ...	1727	17	10 » »	0
Godhavn	669	12	18 » »	1
Kutdligssat ...	1343	4	2.9 » »	1

A large majority of the hospital patients, 46, were between 5 and 15 years of age; 23 boys and 23 girls. Seven were under 15 years of age, 11 were between 15 and 20 and 8 between 20 and 25. Only 3 were over 25 — 3 women at the age of 35, 39 and 51.

The distribution of paralysis and, to some extent, the degree of severity are depicted in Table 2. Twenty-two of the 52 paralytic cases had severe paralysis in all four extremities as well as respiratory paralysis, of these 11 died.

The paralytic and fatal cases, distributed according to age and sex, appear from Table 2.

Four of the women were pregnant and all four had paralysis. One, who was in the fourth month of pregnancy, died in the course of three days. The other three, who were in the 3rd, 5th and 7th month of pregnancy, respectively, all gave birth naturally. The children had no signs of poliomyelitis.

Table 2.
The localization of paralysis in 85 patients from Diskobugten.

	District of Egedesminde	Other 3 districts
Paralysis of 1 upper extremity.	5 patients	8 patients
Paralysis of 2 upper extremities	1 »	0 »
» of 1 lower extremity.	9 »	4 »
» of 2 lower extremities	4 »	3 »
» of 1 upper & 1 lower extremity	3 »	2 »
» of 3 extremities	2 »	2 »
» of 4 extremities	22 »	8 »
Respiratory paralysis	21 »	10 »
Facial paralysis	3 »	4 »
Abdomen only	2 »	2 »
Neck muscles only	1 »	

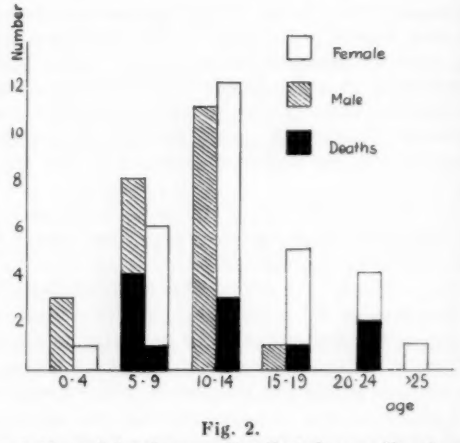

Fig. 2.
Paralytic and fatal cases distributed according to age and sex. (In Egedesminde).

In the most severely attacked villages, we have attempted to calculate the percentage of children who were stricken. The church books record how many children under 12 years were in the various villages. When we accordingly calculate the disease rate from the definitely established polio cases, that, is, those hospitalised who showed pleocytosis, a minimum numbers is obtained. In the three largest villages with 491 inhabitants in all, there were 181 under 12 years of age; out of these, 26 had polio, i. e. 14 %.

In its further course in Diskobugten, the epidemic, on the whole, was less serious, even though there were several severe cases (see Table 1).

In the beginning of September, 5 weeks after the first polio case had occurred in Egedesminde, the epidemic broke out in the Jakobshavn district, where it lasted four weeks. There were many cases of slight illnesses suspicious of polio, and 17 paralytic cases in all, of which 3 from the southern district had already occurred in August but were not recognised before later examinations. The frequency of paralysis was 10 per thousand. No deaths occurred.

In the Godhavn district, the epidemic began

the 25th of September and lasted seven weeks. Also here there was a large but unknown number of polio suspected cases. The frequency of paralysis was high: 18 per thousand, and one death occurred when a child in one of the villages died with signs of respiratory paralysis before help arrived.

In the district of Kutdligssat, polio was found in the town itself where 7 cases occurred, all in the course of one week. Four were severely paralytic, giving a frequency of paralysis of 2.6 per thousand. A 16-year old boy died of respiratory paralysis. Two of the patients who became paralysed had been living in the hospital before contracting the disease: one was two years old and had tuberculosis, while the other was a 16-year old midwife in training.

Thus, there were 33 patients with paralysis from the three districts: 19 male and 14 female patients. The distribution according to age shows that 4 were under 5 years of age, 5 between 5 and 10, 8 between 10 and 15, 11 between 15 and 20, and only 5 between 20 and 24 years. That means that only 5 were born before the epidemic of 1933—34. There were no non-paralytis cases over 24 years of age.

Two women were pregnant, both in the 3rd to 4th months and were stricken only slightly.

The localization of the paralytic cases appears from Table 2.

Of the 10 patients with respiratory paralysis, 2 died, as mentioned.

It has not been possible to find definite means of contagion or disease spreaders. The epidemic broke out after the arrival of the first ship in the spring, but one cannot know if there were people infected with polio on board.

On the basis of the considerable susceptibility of the population and the apparently good possibilities for isolation, quarantine measures were attempted. Among other things, the entire Egedesminde district was closed off from the other districts from the 14th of May to the 1st of August, so that only those ships which had supplies for the district entered Egedesminde which became the last harbour before Ivigtut or Copenhagen. It was not deemed possible, though, to shut of all the villages completely during the summer months when uncontrolled sailing with small boats takes place between the villages.

The epidemic spread to the northernly districts also, and it is probable that one can seldom accomplish little more than delay a new outbreak with such precautions when it concerns polio.

Quarantine measures, at any rate, mean a great deal in Greenland, since because of the considerable distances involved in the Greenlandic medical districts, it would be difficult — not to say impossible — for the health authorities to fight extensive epidemics all over Greenland at the same time.

In the Umanak district, some interesting observations have been made in regard to the behaviour of the polio epidemic in one particular area which is isolated for five to six months of the year because the ice conditions hinder all sailing and where definite quarantine isolation of each of the populated places is possible.

C. F. Øllgaard, doctor of the district, reports (7): the last ship from Denmark before the arrival of winter came to Umanak in the beginning of November, 1952, and there were no persons suspicious of being infected with polio on board. The 21st of November, 1952, polio occurred in Godthaab, and the same day a little coastal ship left for Umanak. Besides the crew, there was a family on the ship who had had contact with the population in Godthaab. The ship was held in quarantine for two weeks in another town and arrived in Umanak the 13th of December, 1952. None of those on board had symptoms of polio.

The 22nd of January, 1953, that is, 40 days later, the first three polio cases occurred in the town of Umanak — three boys with paralysis of the lower extremities. They had all had contact with the family who had arrived from Godthaab. From the first day, the town was shut off from the rest of the district.

The day before the first cases occurred in Umanak, that is, the 21st of January, two men came to town on a visit from the village of Ikerasak, which is 40 kilometer from the town. This was the only contact there had been since the end of November between the town and the district.

On the 9th of March, 1953, 48 days after contact with the infected area, the first cases of polio appeared in Ikerasak.

While Umanak, as already mentioned, had been shut off from the rest of the district, there had been frequent connections with sleds between the various villages and settlements, and during the following weeks, the polio epidemic spread to the various settlements where the epidemic did not stop until the beginning of June.

It can be added that in the Umanak district, with 1500 inhabitants, there was a fairly high frequency of the disease. The rate of paralysis was 14.6 per thousand, that is, 22 paralytic cases, though essentially light cases. There was one death.

Partly because of difficulties of storage and of transportation, it has not been possible to establish the presence of antibodies in the population. On the other hand, Virus Type 1 has been found in occasional feces examinations, the same type found during the polio epidemic in Denmark of 1952—53 (5).

THE TREATMENT

With the violent onset of the polio epidemic and the daily admissions to the hospital in Egedesminde, the problem of caring for and treating the patients rapidly arose.

As soon as the epidemic had broken out, the

Greenland Department immediately sent two physiotherapists and one nurse by plane. Later on, two physicians, two physiotherapists and seven nurses were likewise sent by air. The last group reached Greenland just before the culmination of the epidemic when the hospital was already overfull, and the many admissions had brought about a situation whereby, in several cases, two severely paralysed patients were sharing a bed.

After a few days, two emergency hospitals, which functioned exceedingly well, were set up in the nearby high school and elementary school, thanks to the splendid efforts of the local population. After this it was possible to give the patients the necessary care and observation. The largest number of patients was 48, of which the majority were paralysed.

The most difficult problem was the treatment of those with respiratory paralysis in the acute stage. It meant a great deal that the patients, even from the most distant villages, were rapidly brought to the hospital and that the paralysis, in most cases, developed during the first 24 hours after admission, so that it was really possible to treat those with respiratory paralysis. During the first few days of the epidemic there were several fatal cases. Two Kifa respirators were immediately sent, but with the second wave of hospital admissions these proved to be insufficient, and there was no effective means of treatment before more special materials arrived.

It was planned that, if necessary, tracheotomy with manual positive-pressure ventilation of the lungs would be used, according to the method employed at Blegdamshospitalet in 1952—53, as described by H. C. A. Lassen (6). However, special attention had to be paid to the difficult conditions under which the work had to be carried out in Greenland.

Since it was impossible to add to the supply on hand of bottles with compressed air or oxygen on short notice, it was decided that compressors would be used which could furnish compressed atmospheric air with a pressure of 3—4 atmospheres. Two such compressors were transported and functioned satisfactorily. One compressor could supply air for positive-pressure ventilation for three patients at the same time. When it was required, pure oxygen was added. Positive-pressure ventilation was executed in the same way as at Blegdamshospitalet with manual bag ventilation.

An electric suction apparatus was too unstable and weak for absorbing the secretions from the respiratory tract, so a vacuum pump was set up instead, and 5—6 patients could be served at the same time by connections with the pump.

The difficult problem of finding people to help with the pressure ventilation in such a small community as that in Egedesminde, was solved unexpectedly when four Danes living there and two Greenlandic women undertook the task of carrying it out.

Practically speaking, the treatment of the patients with respiratory paralysis was based solely on clinical judgment at the bedside, as laboratory facilities were very primitive and examinations had to be confined to counting cells in the spinal fluid, haemoglobin estimations, and ordinary urine analyses.

X-ray examinations could not be made in the daily routine with the polio patients who were acutely ill, since the X-ray machine was situated in a building rather far away from the emergency hospital.

As it appears from Table 2, 31 of the 85 paralysed patients had respiratory paralysis of varying degrees of severeness, but all to such an extent that they required special treatment.

Seven died at the hospital early in the epidemic before possibilities for treatment were available. One died while being transported and another died at home before help arrived. After the 24th of June, 22 patients with respiratory paralysis were treated of whom 3 died, at a time when only Kifa respirators were available, but where tracheotomy with positive-pressure ventilation would had been indicated.

The method of treatment for 22 patients with respiratory paralysis was as follows:

1)	postural drainage and possible stomach-tube	9
2)	Kifa respirator only	7 — 3 deaths
3)	Tracheotomy plus respirator..	2
4)	Tracheotomy with positive-pressure ventilation	2 (+ 2 for a short period) — 1 death

All respiratory patients were furthermore treated with lung physiotherapy several times a day in connection with the removal of the secretions through the trachea tube or through coughing exercises.

All these patients received prophylactic penicillin, and one aureomycin besides.

Three patients got atelectases and one of them was bronchoscoped.

The patients upon whom tracheotomy was performed were 9, 12, 12 and 16 years old. Two of them had only a short period of positive-pressure ventilation, but continued afterwards for a longer time in a respirator. Both, however, had normal respiration upon leaving for Denmark, but one had to have positive-pressure ventilation on the trip home. Finally, a 16-year old girl died; in the course of two days, paralysis of all the extremities and respiratory organs developed. She died with hyperpyrexia one and a half days after a tracheotomy had been performed. After being transported to Denmark, two patients required tracheotomy, in connection with infections of the respiratory tract, two and two and a half months, respectively, after the onset of the disease.

Those who were stricken more lightly and needed treatment for a longer preiod of time, were taken to Jakobshavn where two physiotherapists stayed through the winter and gave them treatment. Those patients requiring more extensive treatment were sent to Denmark in September and December on two hospital ships. There were 25 from Egedesminde and 17 from the other districts, a total of 42, who were admitted to Blegdamshospitalet, the Orthopaedic Hospital, and the Hornbæk Therapy Center.

Fifteen months after the epidemic, the following can be reported on their condition from Hornbæk Therapy Center where they are now all receiving further treatment:

With normal functions	9
Slight disability of the hips	4
Disability of the leg or shoulder in children under 6 years of age	5
Supported by one or two braces	14
Severe paralysis of 3 or 4 extremities and believed to be completely or partially disabled	7
Probable permanent and severe reduction of respiratory functions, although respiration is spontaneous	6

The serious polio epidemic in Greenland of 1952—53, which culminated in the district of Diskobugten, has raised a series of problems which must be solved in the years to come.

From the whole of Greenland, 64 patients have been sent to Denmark for more extensive treatment, of these 42 come from Diskobugten. Even though some of these will have normal functions after one to one and a half years of treatment, there will be many cases — an estimated 25 — where the patient will be permanently disabled, e. g., has to use braces or will have reduced functioning of the upper extremities. For all these, the problems of training and employment will be vital. The Greenland Department, in cooperation with the Society and Home for Disabled, has already taken up this question in regard to the older patients. As far as possible, one must try to make them fit for life in Greenland and for procuring an occupation.

SUMMARY

A brief survey is given of the earlier epidemics in Greenland, the first of which probably occurred in 1858.

The polio epidemic of 1953 in Diskobugten, where the last epidemic took place in 1933—34, is described more fully. The Egedesminde district was the hardest hit with a probable disease rate of 42 per thousand and a total of 52 paralytic cases, that is, 18 per thousand of the population, and 11 deaths. The other districts had a total of 33 paralytic cases, between 2.9 and 18 per thousand of the population, and 2 deaths. Of the 85 paralytic patients, 31 had respiratory paralysis and severe paralysis of the extremities.

Sixty percent of the patients were between 5 and 15 years of age. Only 3 were over 25.

The treatment of the patients, and especially the possibilities for treating those with respiratory paralysis under such comparatively primitive conditions, is described.

The condition of 42 of the most severely stricken patients, who were brought to Denmark for further treatment, is given as of 15 months after the epidemic.

Means of contagion have not been proved. Polio-Virus Type I was found in individual patients, the same type found in the epidemic in Denmark of 1952—53.

Finally, the polio epidemic in the Umanak district, which broke out during the winter 40 and 48 days after contact with the infected areas, is described. The frequency of paralysis here was 14 per thousand.

References:

1) *Peart, A. F. W.:* Com. for Public Health 1949, *40:* 405.
2) *Berthelsen, A.:* Meddelelser om Grønland. 1943, Bd. 117, nr. *4:* 151.
3) Information from the medical consultants of the Greenland Department.
4) *Fog-Poulsen, Mogens:* Ugeskr. Læger. 1954, *30:* 1115.
5) Report from *H. von Magnus,* Statens Seruminstitut, Copenhagen, to the Greenland Department.
6) *Lassen, H. C. A.:* The Lancet. 1953, *3:* 37.
7) *Øllgaard, C. F.:* An unpublished report.

INTERHUMAN TRANSMISSION OF ORNITHOSIS

By P. FROM HANSEN and LEIF BØGE SØRENSEN

The transmission of ornithosis from one human to another must be regarded as considerably less frequent than transmission from bird to man.

Confirmed cases of interhuman transmission have not been described previously in Denmark or the Faroe Islands. In Medical Department C, The Copenhagen County Hospital, we had the opportunity of following a small epidemic of ornithosis. It consisted of transmission from a budgerigar (parrakeet) to 2 humans, one of whom transmitted the infection to 4 members of her family. This epidemic will be described in the following.

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In the literature we found accounts of 83 cases of interhuman transmission of ornithosis. Out of these, Elkeles and Barros (1) collected all the cases published until 1931 which are distributed on 13 small epidemics each consisting of from 1 to 8 cases and totalling 32 cases (2—9). The majority of these were nosocomial infections in which nurses, physicians and fellow-patients developed the disease. Several authors emphasize the risk of infection to which fellow-patients and hospital personnel are exposed and the necessity of isolating suspected cases of ornithosis.

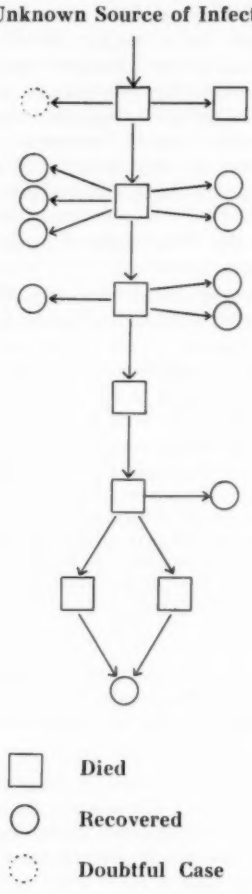
Since 1931, 14 similar cases were described: 3 cases by Eaton, Beck and Pearson (10), 1 case by Aujaleu and Jude (11), 3 cases by Horder and Gow (12), 2 cases by Haagen and Krückenberg (13), 1 case by Vervoart and Ruys (14) and 4 cases by Meyer and Eddie (15). In the article last mentioned, the authors refer to a series of epidemics in various countries during the years 1948—1950, comprizing a total of 161 cases of which 4 cases of interhuman transmission, i. e. approximately 2.5 per cent.

To illustrate the fact that massive infection or prolonged contact is not always necessary, Vervoart and Ruy's case may be mentioned: A Swede was infected by a Dutch friend with whom he had spent some hours in a restaurant in Amsterdam. The Dutchman, who kept exotic birds, had suffered from an influenza-like disease for 2 months. The Swede took ill in Stockholm 14 days after the meeting and the diagnosis of ornithosis was established. In both individuals, the complement-fixation reactions were strongly positive.

The remaining 37 cases originate from two more extensive epidemics in Louisiana and Buenos Aires. The epidemic in Louisiana was described in 1944 by Olson and Treuting (16). It comprized 19 cases among hospital personnel with 8 deaths. The spread of the epidemic appears from the figure below.

It is striking that all of the fatal cases with one exception transmitted the infection to at least one other individual, while the cases which recovered did not transmit the disease. The incubation period calculated from the first day of exposure until the outbreak of the disease varied from 12 to 30 days while calculated from the last day of exposure until the outbreak it varied from 5 to 15 days.

The epidemic in Buenos Aires in 1939 (17, 18) involved 28 patients. Of these 10 males and 3 females died. The epidemic broke out in 3 separate districts but in 2 of these only one case occurred. In the third district, 26 patients took ill. The epidemic commenced by attacking an isolated household in which exotic birds were kept and spread to 3 other families with which this household had contact and ended in the local Jewish hospital where 1 physician, 3 nurses, 2 fellow-patients and 1 visitor were infected directly from the patients. Further, these secondary cases were probably responsible for tertiary cases so that the total number of cases of interhuman transmission amounted to 18 out of the 28.



In several epidemics, among others that in Louisiana, the origin of the first case could not be traced to a bird or another animal. Further, it should be noticed that out of the 88 cases in England in the years 1949—1952 (19), 28 had not been in contact with birds, dogs or cats. Burnet and Smadel (20), among others, propounded the theory that strains of the virus may exist which have adopted man as their primary host. It is obviously difficult to prove the existence of such "humanized" virus strains but that viruses are capable of adapting themselves to different species of animals appears from the fact that viruses belonging to the same group as ornithosis (common antigen) have been isolated from pneumonia in cats and mice, enteritis in calves, infectious abortion in sheep and lymphogranuloma venereum in man.

In Denmark, Moltke (21) accounted for 3 cases of ornithosis in 1932 where the possibility of interhuman transmission was considered. Four

physicians who had been present at the autopsies of diseased parrots conducted by one of the physicians who had, in addition, taken swabs from the throat of a patient with ornithosis, took ill with ornithosis. The physician who performed the autopsy took ill 9 days later; the 3 others took ill on the 10th—12th days after the first day of illness of the former on which day they all were together. The possibility, however, exist that all 4 physicians were infected at the autopsies so that for the 3 of the cases the incubation period is between 19 and 21 days which falls within the limits found in other epidemics.

AUTHORS' CASES

On June 1, 1954 a civil engineer, aged 57 years, was admitted to the Department. On May 6, 54, the patient had taken ill after having been drenched on the preceding day. During the first week of illness, pyrexia of about 39°C. (102.2°F.) was present. Penicillin was administered without effect. On May 14 the patient began to cough and expectorate. Blood sample rendered positive complement-fixation reaction for ornithosis on June 10.

The patient, his wife and 4 out of 5 children presented pulmonary symptoms in April-May 1954. Only the eldest child, a daughter, had been in contact with birds.

Spread of Infection.

A municipal kindergarten had a bird cage with a cock budgerigar (parrakeet) for several years.

On Febr. 1, 1954 the patient's eldest daugther, M., aged 19 years, commenced employment in the kindergarten. Among other tasks she was required to look after the budgerigar and to clean the cage.

In the middle of March 1954, a hen budgerigar was bought.

On April 1 M. began to cough.

On April 13 the hen budgerigar died and a new one was acquired; this bird refused, however, to mate and was exchanged for another which immediately took ill and died on May 1. The cock budgerigar was given away to a boy from whom it flew away.

From April 15 until May 4 M. was on sick leave on account of pyrexia, short sharp cough and copious expectoration.

On April 1 the patient's daughter L., aged 9 years, began to cough; she was seen by her general practitioner who was of the opinion that bronchitis was concerned, and she received 6 injections of penicillin; on April 12 vomiting and whooping developed and the diagnosis was altered to whooping cough; cough-plate examination was not undertaken. On April 24 she suddenly developed pyrexia between 38.5° and 39°C. (101.3°—102.2°F.) with looser and more frequent cough.

On April 17 and 21 the son R., aged 6 years, was vaccinated against whooping cough. On April 28 he developed a cough and was confined to bed for the following week. This case was interpreted as an abortive case of whooping cough.

On May 1 the patient's daughter, A., aged 10 years, developed a cough and flaring up of the recurrent bronchitis to which she was subject. She was confined to bed for 14 days. At the age of 6 weeks, this patient had suffered from severe whooping cough.

On May 6 the father took ill suddenly (see above).

In the beginning of May 1954, the daughter H., aged 14 years, suffered from a cough for 4—5 days but was otherwise symptom-free.

On May 10 the patient's wife developed pyrexia of 38.9°C. (102°F.) and unproductive cough. Coughing continued for the following 4—5 weeks.

Blood samples were acquired from 4 of Miss M.'s colleagues in the kindergarten for the determination of ornithosis complement fixating antibodies. An uncharacteristic, probably positive reaction, was found in Miss H., into whose charge the care of the budgerigars had passed and who also cleaned the cage during the absence of Miss M.. Temporarily, Miss H. had suffered from a dry cough but had otherwise been symptomfree.

All the positive titre reactions showed inhibition zones. These may constitute a source of error as regards the readings of the titres.

DISCUSSION

In several minor epidemics it will prove impossible to distinguish between transmission from bird to man and transmission from man to man, viz. when all the individuals affected have been in contact with the original source of infection, the bird, as for example in M o l t k e's cases. In the epidemic accounted for in the present paper this source of error can be excluded as only one member of the family (M) had been in contact with the bird. In all probability, the dead birds had suffered from ornithosis; the ornithosis reaction in M.'s colleague, Miss H., supports this assumption.

Out of the seven members of the family four, our patient, the daughters A and L. and the son R., probably suffered from ornithosis transmitted by the daughter M.

As our patient had pulmonary symptoms for 3 weeks prior to admission, virus culture was not attempted. We consider the diagnosis in the present cases verified on account of the following criteria: 1) the contact with the diseased birds, 2) the course of the disease and 3) the radiological and serological findings. In this connection it may be mentioned that K. F. M e y e r considers every positive ornithosis complement-fixation reaction with a titre of 30 or more as specific. In The Danish National Serum Institute (Statens Seruminstitut), non-specific reactions have hitherto not been observed. The inhibition zones observed on titration can probably be explained

Table 1.

	Complement-Fixation Reaction for Ornithosis	X-Ray Examination of Lungs	Other Investigations
Patient	10. 6. 54: moderate positive react. 19. 6. 54: positive, titre 60 26. 6. 54: uncharacteristic reaction, probably positive 19. 8. 54: negative	X-ray examination of lungs supplemented by tomography showed fibrosis and calcified processes in both apices. 3. 6. 54: an infiltrate, the size of the palm of a hand situated in the upper lobe lateral to the right hilus. Infiltrate uncharacteristic, possibly interstitial. Control X-ray 19. 8. 54: infiltrate disappeared. 3. 6. 54: both hili enlarged; particularly on left side considerable swelling of lymph glands. Control X-ray 26. 8. 54: hili practically unchanged. Whether enlarged lymph glands were due to ornithosis or the previous tuberculous lesion could not be ascertained.	B. S. R. 2. 6. 54: 60 mm 16. 6. 54: 45 mm 23. 6. 54: 40 mm 26. 8. 54: 26 mm Hb. 91 % W. B. C.: 8,000 Differential count: immature neutrophil leukocytes 2 % polymorphonuclear leukocytes 66 % eosinophil leukocytes 2 % monocytes 10 % lymphocytes 20 % Cold agglutinin titre: nomal Expectorate: no tubercle bacilli Stomach washing: negative Complement-fixation reaction for whooping cough: negative
Wife	12. 6. 54: anti-complementary 17. 6. 54: negative 12. 8. 54: negative	17. 6. 54: No abnormality	
Daughter M.	12. 6. 54: positive, titre 30 19. 6. 54: negative 12. 8. 54: negative	19. 6. 54: No abnormality	
Daughter H.	12. 6. 54: negative 19. 6. 54: negative 12. 8. 54: negative	19. 6. 54: No abnormality	19. 6. 54: Complement-fixation reaction for whooping cough: negative
Daughter A.	12. 6. 54: positive titre 60 18. 6. 54: positive titre 60 12. 8. 54: positive titre 30	18. 6. 54: No abnormality	19. 6. 54: Complement-fixation reaction for whooping cough: very weak reaction of questionable significance
Daughter L.	12. 6. 54: positive titre 30 18. 6. 54: positive titre 30 19. 8. 54: negative	18. 6. 54: No abnormality 19. 8. 54: No abnormality	18. 6. 54: Complement-fixation reaction for whooping cough: pos.
Son R.	12. 6. 54: positive titre 120 18. 6. 54: positive titre 60 12. 8. 54: weakly positive reaction, titre not recordable	18. 6. 54: No abnormality	(vaccinated against whooping cough)

by the fact that the samples were taken during the period of convalescence or the patients may have been poor producers of antibodies.

SUMMARY

The literature concerning interhuman transmission of ornithosis, a total of 83 cases, is reviewed.

An outbreak of an epidemic of ornithosis is described. The epidemic originated from a budgerigar in a kindergarten; an attendant was infected who, in her turn, conveyed the disease to 4 out of the other 6 members of her family. The diagnoses were confirmed by the clinical pictures and the serological reactions.

References:

1) *Elkeles, G. & E. Barros:* Ergebn. d. Hyg., Bakt., Immunitätsforsch. u. exper. Ther. 1931, *12:* 529.
2) *Hegler, C.:* Deutsch. med. Wschr. 1930, *56:* 148.
3) *Kerschensteiner, E.:* Münch. med. Wschr. 1930, 77: 310.
4) *Hamel, C.:* Bull. Office Internat. Hyg. 1930, *22:* 1159.
5) *Günther, F.:* Klin. Wschr. 1930, *9:* 203.

6) *Cosman & Stefanopoli:* Bull. san. de. l'Algerie 1930, nr. *387:* 54.
7) *Sturdee, E. L. & W. M. Scott:* Rep. Public Health Med. Subj., London 1930, nr. 61.
8) *Leichenstern, O. & E. Czaplewski:* Zentralbl. allg. Gesundh. 1899, *18:* 241.
9) *Armstrong, C.:* Public Health Rep. 1930, *45:* 2013.
10) *Eaton, M. D., M. D. Beck & H. E. Pearson:* J. exper. Med. 1941, *73:* 641.
11) *Aujaleu, E. & A. Jude:* Presse méd. 1936, *44:2:* 1094.
12) *Horder, T. & A. E. Gow:* Lancet 1930, *1:* 442.
13) *Haagen, E. & B. Krückeberg:* Veröff. a. d. geb. d. Volksges. 1937, *48:* 381.
14) *Vervoort, H. & A. C. Ruys:* J. Microbiol. & Serol. 1939/40, *6:* 11—21, cited from *K. F. Meyer & B. Eddie:* J. Infect. Dis. 1951, *88:* 109.
15) *Meyer, K. F. & B. Eddie:* Medicine 1942, *21:* 175.
16) *Olson, B. J. & W. L. Treuting:* Public Health Rep. 1944, *59:* 1299.
17) *Loizaga, N. S. & S. Averbach:* Revista de med. y ciencias afines. 1945, *7:* 297, 379, 461, 543.
18) *Garcia, P.:* Prensa med. Argent. 1940, *27:* 2463.
19) *Westwood, J. C. N.:* Proc. Roy. Soc. Med. 1953, *46:* 814.
20) *Burnet, F. M.:* Harvard Univ. Monogr. in Med. and Publ. Health 1945, nr. *8:* 69.
21) *Moltke, O.:* Ugeskr. Læger 1932, *94:* 78.

ANNOTATION

STUDIES ON ORNITHOSIS IN DENMARK

By MOGENS VOLKERT and PREBEN MØLLER CHRISTENSEN

With the object of gaining information concerning the occurrence of ornithosis in Denmark, routine complement fixation tests were carried out in the year 1953 on blood samples from 890 patients, selected mainly among cases of atypical pneumonia. Of these patients, 94 were found to be serologically positive with titers of 30—960. In 15 patients a significant rise in titer could be demonstrated and in 29 the clinico-epidemiological data were so decisive that there could be no doubt that their disease must have been ornithosis. Very little is known about the remaining 50 patients with positive sero-reactions — not enough to justify any reasonable estimation concerning the significance of the complement fixation test. A group of 20 of our patients consisted of persons whose profession involved the handling of birds; 18 of these were serologically positive, 8 showed clinical signs of ornithosis at the time of the examination, whilst the remaining 10 were in good health and their past history was negative in regard to infections that might have been ornithosis.

In most of our cases the ornithosis infection took the form of a relatively mild disease and no deaths occurred. Several of the patients had no lung lesions and the only clinical sign was fever of non-typical character. To the best of our knowledge all the patients showed good response to Aureomycin therapy. Relapses were not uncommon, appearing generally shortly after discontinuation of the chemotherapy. However, all responded again readily to Aureomycin. During the year one patient — a pigeon lover, with a flock of pigeons which was heavily infected with ornithosis — became ill 5 times in 6 months with pneumonia before the cause of the disease was recognized. Another of our patients had a second attack of ornithosis 8 months after the first outbreak of the disease.

In the majority of our cases epidemiological studies revealed that the source of infection must have been birds, particularly cage birds and pigeons, though in some patients no contact with any bird could be found.

Virus isolation attempts were succesful from 2 parrots and 10 pigeons.

Complement fixation tests were positive in 2 out of 7 parrots and in 16 out of 52 pigeons.

The complement fixation tests were performed with »Lygranum« and with an antigen made in this laboratory from a strain of ornithosis virus originating from the American Type Culture Collection, Washington. Comparison of the value of these two antigens was carried out on 52 positive sera and no significant difference could be demonstrated.

In our material the maximum serological titer varies greatly from patient to patient. Most of our patients showed titers of 120—240, but in two cases with unquestionable acute ornithosis the titer did not exceed 30 during an observation period of several weeks. In four cases we found titers of 960. The complement fixing antibodies in the blood of our patients were found to persist for a very long time. In only two cases did we find that the sero-reactions became negative within two months and in these two patients the original titer was only 30. In all others the sero-reaction remained constant or showed a moderate fall in the course of time. Thus we have one patient who still had a titer of 120 13 months after the infection and another who showed a constant high titer of 240 throughout a period of 11 months.

For more detailed information regarding the cases and data mentioned above see:

Ugeskrift for Læger, 1954, *116:* 867, and
Acta Path. and Microbiol. Scand.: in print.

From Statens Seruminstitut, Copenhagen.
Chief: *J. Ørskov,* M. D.

DECOMPRESSION OF THE FACIAL NERVE IN CASES OF HEMIFACIAL SPASM

By KARSTEN KETTEL

Hemifacial spasm is a well defined disease, consisting of tonico-clonic contractions of all the muscles innervated by a single nerve: the facial nerve. The muscles most commonly afflicted are those around the eye and mouth.

Fig. 1.

Cases of hemifacial spasm may be divided into two groups: primary and post-paralytic.

a) *Primary hemifacial spasm*, by some authors termed "cryptogenic" "idiopathic" or "autonomous". In reviewing 106 cases Ehni and Woltman gave the following characteristics of this group: (1) the spasms are of an intermittent, twitching nature, (2) the eyelids on the ipsilateral side are almost always involved, (3) the spasms are usually unilateral and when bilateral they are not synchronous or equal in extent or severity, (4) the spasms may persist in sleep, (5) the patient does not feel any compulsion to make the movement, (6) the patient is unable to stop the movement by exercise of the will, (7) the patient cannot reproduce the movement voluntarily, (8) psychic upsets of any sort, fatigue and voluntary movements of the face, make the condition worse, (9) children do not have hemifacial spasm and (10) the spasms are limited to muscles innervated by the facial nerve.

It might be added that the contractions generally start in m. orbicularis oculi, and during weeks or months they are spreading to the neighbouring muscles and are intensified in strength and duration. I have, however, seen two cases where violent spasms started suddenly and affected all the facial muscles from the start, on the side afflicted. The spasms may be accompanied by vasomotor and secretory disturbances of the same side of the face. Pain is an uncommon symptom.

b) *Postparalytic* or "symptomatic" hemifacial spasm. The cause of the symptomatic hemifacial spasm may be intracranial lesions (tumours, aneurism, meningoencephalitis, injuries) causing irritation of the facial nerve or nucleus, resulting in spasm (O'Donnell). Among Alajouanine & Thurel's 52 cases, 4 were caused by a cerebello-pontine tumour. Most of the postparalytic hemifacial spasms, however, are due to Bell's ischaemic facial palsy.

In a very thorough paper by Williams, Lambert & Woltman the whole problem is surveyed. They state that "the available evidence suggests that the hemifacial spasm which follows Bell's palsy and the hemifacial spasm called "cryptogenic" are clinically indistinguishable except by history, because muscular weakness and contracture are equally prevalent in both. The evidence also indicates that the etiology of each condition may be the same".

PATHOLOGY

Williams, Lambert & Woltman in their paper state that two hypotheses have been offered as to the cause of the primary as well as the postparalytic hemifacial spasms: (1) the first suggests that hemifacial spasm is a central effect due either to degeneration of cells in the rolandic motor cortex or to degeneration of cells of the facial nucleus in the medulla; (2) the second hypothesis is that hemifacial spasm is a disorder of the lower motor neuron.

In reviewing the experimental work performed by many authors to elucidate this question, Williams, Lambert & Woltman conclude that the hypothesis of the central origin of these spasms is completely untenable. The facts derived from the clinical and experimental investigations "make it seem highly probable that the lesion causing hemifacial spasm lies somewhere between the facial nucleus and the stylomastoid foramen".

As already outlined, the primary (cryptogenic) and

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the postparalytic hemifacial spasm are clinically indistinguishable except by history. At present there is a general agreement that Bell's palsy is due to ischaemia (Worms & Chams, Audibert et al., Kettel, Hilger, Cawthorne, Sullivan, Findlay, Jongkees, Botman). In addition to the alterations found in the facial nerve (haemorrhagic streaks in the sheath and a constriction of the nerve at the stylomastoid foramen wih oedema proximal to this point) I have, in 20 of 108 cases (latest check-up, 1954), found bony necrosis and exudation in the the mastoid cells, especially near the stylomastoid foramen, findings which have been confirmed by European surgeons (Jongkees, Hall, Flodgren, Skoog), and drawn the conclusion that Bell's palsy is a pathological entity, the "dysregulation" of the circulation, in most cases affecting only the nutrition of the nerve as the most susceptible tissue, from which an ischaemic paralysis arises, but in others affecting also the nutrition of the facial canal and the mastoid cells, causing bony necrosis.

Williams, Lambert & Woltman on this basis state as follows: "In considering the hypothesis that vasospasm may be the fundamental cause of Bell's palsy, it may be pointed out that vasospasm sufficient to produce necrosis, when it has been observed elsewhere in the body, appears to be associated with tissue injury of a sort which maintains a reflex to the affected vessel. It might be considered reasonable to suppose, therefore, that in Bell's palsy and primary or cryptogenic hemifacial spasm, even though vasospasm might play a part in producing the lesion, there might be a still more fundamental tissue lesion tending to set up a reflex, producing the vasospasm. On this basis it occurred to one of us (Woltman) that the fundamental lesion in both hemifacial spasm and Bell's palsy might be a fibrous constriction of the sheath of the facial nerve somewhere in its course through the temporal bone, but probably in close relationship to the stylomastoid foramen."

OPERATIVE FINDINGS IN HEMIFACIAL SPASMS

Decompression of the facial nerve was performed by Williams, Lambert & Woltman on 7 patients suffering from hemifacial spasm and one from blepharospasm, with the following findings: a) in two cases the nerve and the nerve sheath were macroscopically normal; b) in one case a fibrous nodule about 2 mm. across was found attached to the inner sheath at the stylomastoid foramen; c) in 5 cases the nerve sheath near the stylomastoid foramen was tough and resistant to slitting.

My own material consist of 10 cases of hemifacial spasm (7 primary and 3 postparalytic, all women) and one of blepharospasm. The idea of decompression in these cases occurred to me (1943) because I had seen 3 cases of Bell's palsy who had spontaneously made a partial recovery marked by contracture which disappeared in immediate conjunction with the operation. I thought that if the contracture was due to a constant irritation of the facial nerve within the Fallopian canal (Grindstein's theory) which could be relieved by decompression, this might be well worth trying in cases of hemifacial spasm (fig. 2).

The prompt results which followed my first decompressions made me operate on 11 patients in quick succession. The therapeutic disappointments were to follow later. All the patients have been submitted to a very thorough medical, serological, neurological, otological, ophtalmological and radiological examination expressly to exclude intracranial lesions.

Primary hemifacial spasm (7 cases) — All the patients were women, 28—70 years of age, the contractions were severe and had in 6 cases

Fig. 2.
A: Bell's palsy of seven months' duration on the left side, with contrature. B: appearance of the patient the day after the operation; the contracture disappeared immediately after decompression without the slightest impairment of the mobility. C: 2½ years after decompression.

lasted between 3 and 9 years. The last patient was operated only one month after onset, because the contractions were violent, and because my results from decompression at that time seemed to be excellent.

The operative findings were as follows: a) in 3 cases the mastoid cells and the nerve looked perfectly normal; I must however admit that not until I read years later (1952) the paper by Williams, Lambert & Woltman, was my attention especially drawn to alterations of the nerve sheath; b) in one case a circumscribed nodule looking like a dilated vessel, 2—3 mm. across, was seen within the nerve sheath just proximal to the stylomastoid foramen; in more than 250 operations on the facial nerve according to the procedure of Ballance & Duel, I have never seen anything similar; c) in one case, operated one month after the onset, the nerve was distinctly oedematous, and bulged through the slit in the nerve sheath, exactly as noted in many cases of Bell's palsy; d) in one case the nerve sheath was very tough and resistant to slitting; e) in the last case pronounced alterations were encountered.

This patient, a 51-year-old woman, had for 8 years suffered from increasing hemifacial spasms resistant to all medical therapy; apart from this a thorough examination showed nothing abnormal; there was no facial weakness, she had never suffered from ear troubles, and the drums and hearing were perfectly normal. The superficial mastoid cells were completely normal but the bone between the posterior osseous wall of the external auditory meatus and the sigmoid sinus, as well as the mastoid tip, was completely necrotic; the wall of the facial canal was very soft and was opened as far as the lateral semicircular canal in a few minutes; the nerve was distinctly oedematous; near the stylomastoid foramen the mastoid cells contained a yellow transudate or exsudate; neither pus, nor granulation tissue were found; microscopy showed granular bony decay and bony necrosis, while on direct examination of the fluid no bacteria were found. A facial weakness followed the operation but disappeared soon, and for 10 months the patient was completely free from hemifacial spasms and synkineses; then they recurred.

One year after the first operation a revision was performed. The bony cavity was normal and in it the facial nerve was lying free; in its proximal half, between the lateral semicircular canal and the stylomastoid foramen, it looked normal; in the distal half, it lay surrounded by connective tissue from which it was freed. This time no facial weakness followed, but the hemifacial spasms were not influenced by the neurolysis. Five years later she was still suffering from severe hemifacial spasm.

Postparalytic hemifacial spasm (3 cases) — All the patients, women 32, 40 and 57 years of age, had suffered from Bell's ischaemic palsy (for 8 months, 9 months and 10 years, respectively); in the last two cases a contracture had developed in addition to severe hemifacial spasm.

The operative findings were as follows: a) in two cases (which had lasted 8 months and 10 years, respectively) nothing abnormal was seen in the mastoid cells or the facial nerve; b) in one case of 9 months duration, in which the patient had never suffered from otitis media, and where the objective examination showed nothing abnormal apart from the hemifacial spasm and a contracture, the superficial mastoid cells were large and clear; the cells between the posterior wall of the auditory meatus and the sinus, on the other hand, were smaller and soft, and the facial canal proper appeared so soft that the decompression was completed in a few minutes; in the part deep to the nerve a cavity larger than a hazel-nut was created after the excochleation, and through this cavity the nerve had been running practically uncovered; the cavity passed deeply toward the jugular foramen; the nerve sheath was slit open, and the nerve bulged distinctly.

Blepharospasm (man 19 year old) — Nothing abnormal was found in the mastoid cells or in the nerve.

To sum up Williams, Lambert & Woltman's cases, the nerve and nerve sheath were normal i 2 cases, while in 6 pathological alterations were found near the stylomastoid foramen. Concerning my own cases, it is seen that the mastoid cells in 9 cases were normal, while in 2 cases they were the site of severe alterations as described above. The facial nerve was normal in 6 cases, while in 5 cases alterations of varying nature were encountered at or just above the stylomastoid foramen.

COMMENTS

The most interesting results of decompression in cases of hemifacial spasm are (a) the pathological alterations, and (b) the disappearance of associated movements of the facial muscles.

a) *Pathological findings.*

Three clinical entities are known in which all the subjective and objective findings are strictly confined to the region innervated by the facial nerve: (1) Bell's palsy; (2) Melkersson's syndrome, which consists of a peripheral facial palsy, exactly like Bell's ischaemic, but combined with angioneurotic oedema of the lips and cheek, and sometimes with a lingua plicata; and (3) hemifacial spasm.

In Bell's palsy and Melkersson's palsy the weakness is the essential feature, the postparalytic spasm a rare and secondary phenomenon. Synkineses, more os less pronounced, are, however, a practically constant symptom. In *hemifacial spasm* contractions are the dominant symptom, weakness being slight or completely lacking. By decompression, however, I have found examples of exactly the same pathological alterations in the mastoid cells near the stylomastoid foramen and in the facial nerve.

1) *Bell's palsy:* In 20 out of 108 cases I have, as already described, found bony necrosis in the tip of the mastoid cells near the stylomastoid for-

amen and in some cases exudation into the cells. In 26 cases the wall of the facial canal, normally hard like ivory, was more or less soft, and in the extreme cases completely necrotic. The alterations were exactly like those described above in 2 cases of hemifacial spasm. In 56 cases the nerve was distinctly oedematous, in 3 (long standing palsies) atrophic.

2) *Melkersson's syndrome:* In 10 cases the mastoid cells were normal, in one a limited area of bony necrosis was found near the stylomastoid foramen. The nerve in 3 cases looked normal, in 8 it was oedematous and bulged distinctly after slitting of the sheath. The last case (described in detail previously) was quite exceptional:

In a patient 30 years of age, suffering from a complete peripheral palsy of 3 months duration, decompression revealed the following alterations: "The superficial cells are large and glossy. In the depth around the antrum and in the cells between the posterior wall of the auditory meatus and the sinus the bone is soft, increasingly so toward the part around the stylomastoid foramen. The entire facial canal is completely necrosed in its distal half, and the remaining part is opened. Neither knife nor scissors were used, but the nerve sheath is seen to be open, and from it a highly oedematous piece of the nerve bulges out, being conically pointed downward and of a grayish pink color. The point of the nerve, however, is of a much darker red color than the remaining part. The piece of the nerve projecting from the opening in the sheath is 7 mm. long. It is resected and examined microscopically. A nerve transplant taken from the ilioinguinal nerve is grafted" (and the mobility reappeared ten months later). Microscopy showed: "Oedema of the nerve trunk, fibrinous exudation, fresh haemorrhage and marked degeneration of axiscylinders and medullary sheaths, a small vein containing organized remnants of a thrombus, and between nerve and its sheath an accumulation of fluid consisting of oedema and fibrin". (Svend Petri). There is not the slightest doubt that these alterations are due to disturbance of the blood supply. Neither micro- nor macroscopie signs of infection were encountered.

3) *Hemifacial spasm:* As described above, 2 cases were encountered in which very pronounced alterations of the mastoid cells were found. In 3 cases the nerve was oedematous. Everything points to ischaemia being the cause of the alterations; in no case were signs of infection found.

Thus Bell's palsy, Melkersson's syndrome and *hemifacial spasm* have clinical as well as pathological features in common, and are all probably due to vascular disorders near the stylomastoid foramen.

b) *The disapperance of associated movements of the facial muscles.*

This is considered by Williams, Lambert & Woltman the most interesting result of decompression in hemifacial spasm.

Just like these authors I have noted that the spasm invariably disappeared in immediate conjunction with the operation even if no postoperative weakness of the facial muscles followed (2 cases). In cases in which a more or less pronounced facial palsy resulted from decompression, the spasms, when they recurred, appeared months after the postoperative palsy had disappeared.

As pointed out by William, Lambert & Woltman, associated movements have generally been attributed to either branching or misdirection of nerve fibers during regeneration. This explanation of associated movements according to Williams, Lambert & Woltman is not in harmony with the disappearance of the synkineses after decompression, which should be explained on the basis of a reversible physiological mechanism, rather than an irreversible anatomical one. "A possible mechanism of hemifacial spasm which appears to fit the observation which have been made assumes the occurrence of spontaneous discharges of interaction or "cross firing" of nerve fibers in the facial canal", illustrated in fig. 3. "There is no conclusive evidence that the mechanism just described is the mechanism of hemifacial spasm. It is only true that, thus far, our observations have not been inconsistent with this concept. The relief of hemifacial spasm by decompression of the facial nerve suggests that the genesis of the synkinesis and spasm may lie in the facial canal".

In discussing the paper cited, Lathrop ex-

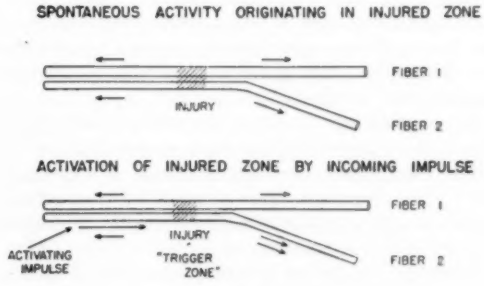

Fig. 3.

A possible mechanism of hemifacial spasm. Fibers 1 and 2 represent axons of the facial nerve innervating the upper and lower parts of the face, respectively. Local injury to the nerve by compression and ischemia in the facial canal is indicated by cross hatching. Upper diagram: spontaneous discharge of impulses (the arrows) from the injured zone produces spasm or contracture. Lower diagram: during interparoxysmal periods, nerve fibers in the injured zone are hyperexcitable. The action current of a "voluntary" impulse in fiber 2 acts as a trigger to the injured zone, causing a discharge of impulse in both fiber 1 and fiber 2, whereas only the impulse in fiber 2 was willed. Synkinesis results from this interaction between nerve fibers. The effects of antidromic impulses in motor fibers and of impulses in sensory fibers have not been considered in this discussion. (Reproduced from Woltman, Williams & Lambert, by courtesy of Dr. Williams).

pressed his belief that it is injury to the facial nerve, which has resulted in minor temporary depression of function, which affords the patient the relief from the hemifacial spasm that he obtains.

I am not disinclined to agree with Lathrop. The explanation why spasm and synkineses do not return immediately with return of function after the postoperative weakness following decompression, may be, as stated by Williams, Lambert & Woltman, that after all axon dichotomy is the explanation of associated movements, and that impaired nerve fibres are more vulnerable to additional injury, so that if only a few axons were involved in this process these might be destroyed by the trauma of surgery or at least be the last to recover.

THERAPY

Only the treatment of those cases in which medical and neurological therapy had failed shall be discussed here.

Is decompression of the facial nerve the right operation for resistant and severe cases of hemifacial spasm? Williams, Lambert & Woltman state that the operation of neurolysis for hemifacial spasm has proved somewhat disappointing in that there is a tendency for recurrence of the spasm after a time, although most patients report considerable relief from the procedure.

Williams in a recent personal communication to O'Donnell, advised him of the final results in 9 cases, all observed over two years. Five cases had had a short period of relief but the spasm then reappeared as severely as before the operation. This gives three good results and one fair out of nine.

My results are completely in accordance with this statement. All the patients operated on 8—10 years ago have been re-examined 2—10 years after the operation. The number in parenthesis indicates the number of years after the operation when I saw the patient last.

Among 7 cases of primary hemifacial spasm, one patient had recovered completely (10 years); 2 patients were only troubled by faint contractions, and only when they were in a nervous state of mind (8½—10 years); 4 patients were relieved of the spasms for ½—1 year, then the recurrence took place and the condition is as bad as before operation (3—5—10—10 years). The same applies to the man with blepharospasm (9 years). Among the 3 cases of postparalytic spasm, 2 were only troubled very little by contractions (2—10 years), but in one case the condition was as bad as before (2 years).

SUMMARY

Among 11 patients a complete cure was obtained in one case, a fair result in 4 cases, while in 6 cases the effect of the operation has only been temporary and full recurrence has taken place. Even if decompression has thus resulted in a few recoveries and improvements, the results in the majority of cases have been disappointing.

Everything points to hemifacial spasm being due to a disorder of the lower motor neuron. Intracranial lesions in the vicinity of the facial nerve are known to have resulted in irration and spasm. It may be perfectly true that the majority of cases of hemifacial spasm are due to a lesion, the nature of which may vary, in the Fallopian canal near the stylomastoid foramen, not least the postparalytic following Bell's palsy.

But the disappointing results of decompression seems to indicate that at the time of operation irreparable damage to the nerve has in the majority of cases been already done. Consequently I gave up decompression in cases of hemifacial spasm some years ago.

Good results from injections of alcohol into the nerve have been reported (Jessen) but I prefer selective sections of the branches to the muscles involved as described by German and Greenwood.

References:

Alajouanine, Th. & R. Thurel: Paris Méd., 1939, *114:* 165.

Audibert, V., C. Mattei & A. Paganelli: Presse Méd., 1936, *44:* 1049.

C. Ballance & A. B. Duel: Arch. Otolaryng., 1932, *15:* 1—70.

Botman, J. W. M.: Over Verlammingen van de Nervus Facialis van Intratemporale Oorsprong. Thesis Amsterdam 1954, Uitgeverij Excelsior's Gravenhage.

Cawthorne, T.: a) Laryngoscope, 1946, *56:* 653; b) Lancet, 1952, *I:* 1219.

Ehni, G. & H. W. Woltman: Arch. Neurol. a. Psychiat., 1945, *53:* 205—211.

Findlay, J. P.: Facial Paralysis. Angus & Robertson Ltd., 89 Castlereagh St., Sydney, 1950.

Flodgren, E.: Contributions to the surgery of facial palsy. Acta Otolaryng., suppl. 74, pg. 188, 1948.

German, W. J.: Surgery, 1942, *11:* 912—914.

Greenwood Jr., J.: J. Neurosurg., 1946, *3:* 506.

Grindstein, A.: Zentralblatt f. Hals-, Nasen- u. Ohrenh., 1926, *8:* 299.

Hall, A.: Arch. Otolaryng., 1951, *54:* 475.

Hilger, J. A.: Laryngoscope, 1949, *59:* 228—235.

Jessen, H.: U. f. L., 1940, *42:* 1081.

Jongkees, L. B. W.: Acta Oto-Laryngologica, vol. 44, 1954 (july—aug.), Fasc. 4, p. 336.

Kettel, K.: a) Arch. Otolaryng., 1947, *46:* 427; b) Arch. Otolaryng., 1947, *46:* 341; c) U. f. L., 1953, *10:* 353.

Lathrop, F. D.: Discussion of paper by Williams, Lambert & Woltman.

Melkersson, E.: Hygiea, 1928, *90:* 737.

O'Donnell, M. C.: Ann. Otol., Rhin. a. Laryngol., 1953, *62:* 969.

Skoog, T.: Personal communication.

Sullivan, J. A.: Laryngoscope, 1952, *62:* 449.

Williams, H. L., E. H. Lambert & H. W. Woltman: Ann. Otol., Rhin. a. Laryng., 1952, *61:* 850.

Worms, G. & Chams: Société d'ophtalmologie de Paris. 1931, February 21st.

Woltman, H. W., H. L. Williams & E. H. Lambert: Proc. Staff Meet., Mayo Clin., 1951, *26:* 236—240.

DISTURBANCES IN THE ACUITY OF SMELL FROM RESPIRATORY ANOMALIES

By KAJ ZILSTORFF-PEDERSEN

For examination of the sense of smell two procedures may be employed. One is to let the experimental subject inhale or sniff the smelling substance; the other consists in blowing the smelling substance up towards the olfactory region while the subject holds his breath.

The former procedure implies some sources of error that may convey an impression of impairment of the smelling sense even though the receptor organ and its central connection function quite normally. This will be illustrated by the following three case histories:

Case 1.

Man, 18, with total facial paralysis appearing during an acute exacerbation of chronic otitis media. Smelling had been greatly impaired on the paralyzed side since the appearence of the facial palsy.

When tested he could faintly smell citral, menthol and oil of turpentine on the paralysed side but not benzaldehyde, musk-ketone, coffee, xylol or phenylethyl alcohol. On the other side the smell of all eight substances was claimed to be quite distinct. Rhinological examination showed no abnormality, and the air passage through the nose appeared to be normal. On olfactometry *ad modum* Elsberg the threshold value for coffee was found to be 3 cc. on either side while the threshold value for citral was 4 cc.

Case 2.

Woman, 60, with gradual bilateral decrease of passage through the nose for the last 10—12 years. Obstruction was now nearly complete with total abolition of the sense of smell.

Examination revealed a total membranous occlusion of both choanae. The nasal cavity contained some mucoid secretion but appeared otherwise normal. The patient was completely unable to breathe through the nose and to smell any of the eight test substances employed. Olfactometry *ad modum* Elsberg showed the threshold values for coffee to be 5 cc. on either side, whereas the threshold value for citral was 4 cc. on either side.

Case 3.

Man, 53, admitted for subacute respiratory difficulties.

Laryngoscopy revealed bilateral paralysis of the recurrent nerve, with both vocal cords in the paramedian position. As the glottis was only 1—2 mm. wide and the patient had difficulty in breathing, tracheotomy was performed. The cause of the paralysis of the recurrent nerve remained obscure.

On examination of the smelling sense five months later when both vocal cords were still immobile, and the patient therefore still had a tracheotomy tube, the sence of smell was claimed to be impaired, with only a faint smelling sensation for citral and menthol, on both sides; none of the remaining six substances could be smelt.

On olfactometry *ad modum* Elsberg, the threshold values for coffee as well as citral were found to be the same on both sides: 5 cc. and 9 cc., respectively.

On reexamination, 1 year later, both vocal cords were movable to a normal extent, and the sense of smell was stated to be normal and the patient was now able to differentiate all the eight test substances.

COMMENTS

In all three cases the patients themselves stated that their capacity for smell had been greatly reduced or abolished. Further, this was indicated also by sniffing test or forced inhalation of the various test substances. On blast injection *ad modum* Elsberg, however, it turned out that the impairment of the smelling acuity was not due to changes in the perception itself (essential impairment of smell) but to changes in the capacity of the patients for transport of the smelling substance from the nostrils to the respiratory region (respiratory or mechanical disturbances of the smell).

As it is important to arrive at an exact etiological diagnosis as far as possible in disturbances of the smelling sense it is necessary to the distinguish between essential and respiratory causes. According to Hennebert, the mechanical or respiratory disturbances of the smelling sense may be brought about by:

1) Anomalies of the nostrils or choanae.
2) Total or relative occlusion of the nasal cavity.
3) Impermeability of the olfactory region.
4) Respiratory disturbances independent of the nasal cavity.

Ordinary rhinological examination, including the inspiratory and expiratory aspects, may often offer some suggestion as to whether a possible impairment of the smelling sense be due to some mechanical hindrance. This applies to all changes presenting rather voluminous processes in the nasal cavity as well as to pronounced deformities of the nostrils or choanae. In other cases it may

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be more difficult directly to realize the significance of the mechanical factor. In normal inspiration through the nose the air current does not get higher up in the nasal cavity than to the concha media. Nevertheless, various odours will be noticed during ordinary breathing through the nose, and this may be due to the circumstance that the air in the smelling substance diffuses up into the olfactory region or the complex structure of the nasal cavity gives rise to eddy currents reaching the olfactory region. The latter seems the more probable because diffusion is too slow to account for the rapid perception of an odour we normally experience (Moncrieff). When on normal inspiration we perceive an unusual odour we either inhale deeply or sniff, and only then do we get the full impression of the odour, corresponding to the fact that only with these procedures is the sense of smell stimulated so strongly that a given odour can be characterised fairly definitely or possibly identified.

In 1870 Ogle stated that there are two modes in which we purposely smell at a subtance. In one we close the mouth and then take a long deep inspiration through the widely dilated nostrils so that the air current charged with odour sweeps over the entire internal surface of the nose, the olfactory region included, and excites a sensation. In the second we close the mouth and draw in the air by a rapid succession of shallow but forcible »sniffs«, the nostrils actually contracting at each sniff. The contraction does not include the whole anterior opening but only its posterior portion. At the same moment the cartilaginous sides of the nose undergo lateral compression by action of the compressor naris muscle, hereby closing the usual respiratory channel and forcing the air through the olfactory channel.

Ogle reported the case of a man suffering from double facial palsy, complete on the left side and almost complete on the right. On the left side the patient could hardly smell the strongest odours, while on the right he could smell somewhat better though still very imperfectly. Ogle explained this impairment of the smelling sense as resulting directly from the facial paralysis. On deep inspiration no sensation of smell is obtained because the facial paralysis interferes with the active dilatation of the nostrils, and in sniffing tests the facial paralysis prevents the lateral compression that is required to close the respiratory channel.

In his original description of peripheral facial paralysis, Charles Bell mentioned impairment of the smelling sense as a concomitant symptom, it is true, but as far as we can see, Ogle was the first to realize why the sense of smell here was impaired.

By tracing of electrograms from the muscle of the wings of the nose, Dishoeck (1937) has shown that the function of these muscles consists in tonic fixation of the width of the internal ostium together with the possibility of its active dilation. This tonicity or capacity for dilatation is abolished by total facial paralysis — as is illustrated by Dishoeck in one of his patients.

In case 2 the total occlusion of the choanae excluded the possibility of any air passing through the nose on respiration and, consequently, no odorous air current could reach the olfactory region and thus stimulate the smelling sense.

In patient no. 3, it seems rather reasonable to think that, on account of the tracheotomy and the marked constriction of the glottis, no inspiration could be sufficiently strong to produce the air current necessary for stimulation of the smelling sense. (Kindler & Ulich).

CONCLUSION AND SUMMARY

The smelling sense was claimed to be markedly impaired or completely abolished in the case of three patients, one of whom was suffering from unilateral total facial paralysis, another from bilateral complete occlusion of the choanae and the third from bilateral recurrent nerve paralysis requiring tracheotomy. Blast olfactometry *ad modum* Elsberg showed normal threshold values on both sides. Thus, the impairment of the smelling capacity was not due to any primary affection of the smelling sense.

It is of diagnostic as well as prognostic significance to know whether the receptor organ for smell (or its central connections) is normal and it is advisable, therefore, to employ Elsbergs blast olfactometry for examination of the smelling sense in patients who claim this to be impaired. In this way it is practicable to avoid some of the sources of error in estimation of the smelling sense owing to alteration of the air passage through the nose.

References:

1) *Bell, C.:* An Exposition of The Natural System of The Nerves of The Human Body. London 1824.
2) *Dishoeck, H. A. E.:* Acta oto-laryng. 1937, *25:* 285.
3) *Elsberg, C. A.* and *I. Levy:* Bull. Neurol. Instit. N.Y. 1937, *4:* 5.
4) *Hennebert, P. E.:* L'Olfaction. Les Editions "Acta Medica Belgica", 1953.
5) *Kindler, W.* and *K. Ulich:* Archiv Ohr. usw. Heilk. 1952—53, *162:* 512.
6) *Moncrieff, R. W.:* The Chemical Senses. London 1951.
7) *Ogle, W.:* Medico-Chirurgical Transactions. 1870, *53:* 263.
8) *Proetz, A. W.:* Applied Physiology Of The Nose. Saint Louis 1941.

ENZYMATIC ADAPTATION I MAN

THE PHOSPHORYLASE ACTIVITY OF SUBCUTANEOUS TISSUE FROM NORMAL SUBJECTS AND FROM OBESE PATIENTS BEFORE AND AFTER DIETARY TREATMENT

By N. R. HAAGENSEN

Enzymatic adaptation has been defined as a change in enzymatic activity following changes in the composition of the surrounding medium (Spiegelman 1950). This biological mechanism is a wellknown phenomenon which has been studied particularly in microorganisms — bacteria and yeast fungi — and, recently, in experimental animals (Mandelstam & Yudkin 1952).

Experiments on the phosphorylase of subcutaneous adipose tissue, reported in this paper, show a similar change in human beings.

MATERIAL AND METHODS

The obese patients were women admitted to Steno Memorial Hospital for treatment of obesity. The overweight ranged from + 30 % to + 140 %, the age ranged from 20 to 53 years. The normal subjects were women of normal weight without signs of metabolic or hormonal disorders, age range 17 to 63 years.

The obese patients were treated with a diet of 1100—1200 cal. a day (60 g of protein, 60 g of fat, and 90 g of carbohydrate), i. e. a low caloric diet with a specially reduced carbohydrate content. No drugs were given.

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Subcutaneous adipose tissue was obtained by biopsy from the coxal region.

Phosphorylase activity was measured according to Cori & Cori (1940) and Green & Cori (1943) on homogenates of the adipose tissue which was treated with petrol ether prior to dialysis. The preparation was carried out in the cold room (4° C.).

Activity measurements are expressed in units and are based on the determination of the first order velocity constant under standard conditions.

Analyses for inorganic phosphate were performed according to Martin & Doty (1949).

N-determinations were made on aliquots of the dialyzed homogenate by a Kjeldahl procedure.

Experiments were carried out shortly after admission and after a period of treatment (20—65 days).

RESULTS

The mean value of phosphorylase activity in the different groups are:

Normal subjects: 3.3 units per mg of protein.

Obese patients before treatment: 11.3 units per mg of protein.

Obese patients after treatment: 4.1 units per mg of protein.

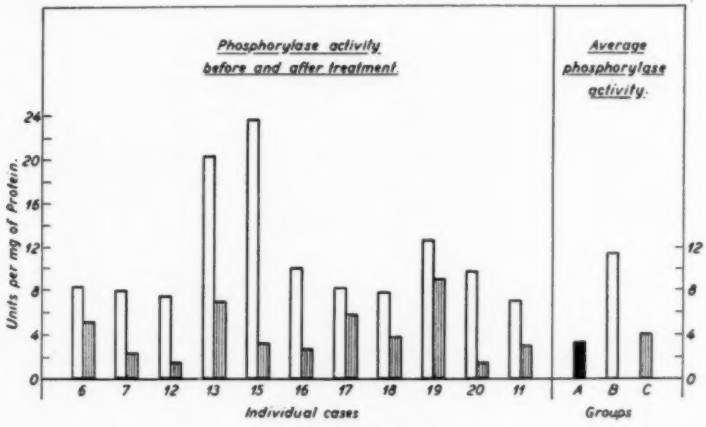

Fig. 1.
Phosphorylase Activity of Human Subcutaneous Tissue.
■ A. Normal subjects.
□ B. Obese patients before treatment.
▥ C. Obese patients after dietary treatment.

In every individual case the enzyme activity decreased during the experimental period. The difference between the enzyme activity before and after treatment is statistically significant.

The findings are illustrated in fig. 1 which shows the phosphorylase activity of the individual patients before and after treatment and the average value of enzyme activity in the groups studied. The marked differences are readily seen.

DISCUSSION

The metabolic activity of adipose tissue has been stressed in a number of recent communications. The fat depots are not essentially inert storehouses of energy as was previously thought, but are constantly involved in a variety of reactions of synthesis, degradation, and interconversions (Schoenheimer 1942).

Among these processes the synthesis of fat from carbohydrate in the peripheral depots call upon a special interest.

Our knowledge concerning the intermediary steps in this synthesis is still scanty. However, a number of studies on the occurrence of glycogen in adipose tissue suggest that this substance is interpretable as an intermediate product in the conversion of carbohydrate to fat (Tuerkischer & Wertheimer 1952; Renold, Marble & Fawcett 1950; Engel & Scott 1950, 1951; Engel 1951; Stetten & Klein 1945).

Glycogen is demonstrable in the fat cells not only in experimental animals but also in human beings (Arndt 1927; Haagensen 1953).

All information at hand suggests that carbohydrate must first undergo glycolysis before its utilization for fatty acid synthesis. Accordingly, the accumulation of glycogen may be regarded as a temporary storage of glucose in the cell — an »osmotic buffer« — and its disappearance which takes place at the same time as the newly formed fat appears (Tuerkischer & Wertheimer 1942) possibly reflects the glycolytic breakdown.

The synthesis of glycogen depends on the action of the enzyme phosphorylase. This enzyme, which in 1943 was crystallized from muscles by Green, Cori & Cori was demonstrated in adipose tissue by Mirski (1942) and by Shapiro & Wertheimer (1943).

In the development of obesity this mechanism may be of some significance as the caloric surplus most often is due to an excess intake of carbohydrates.

In the experiments here reported the phosphorylase activity in subcutaneous adipose tissue proved to be considerably greater in obese than in normal subjects, and this high activity returned to nearly normal values following the treatment.

The changes observed in the enzymatic activity may be explained according to the theory of enzymatic adaptation. During the development of obesity the excess amount of carbohydrate in the food which is ultimately stored as fat in the depots may have caused a prolonged disturbance of the equilibrium of the enzyme systeme and its precursors, the phosphorylated products of glucose being the substrate for the enzyme studied.

During treatment with a diet low in carbohydrate the activity of the enzyme decreased, demonstrating that the equilibrium has been shifted in the opposite direction.

SUMMARY

1. Phosphorylase is demonstrated in human subcutaneous adipose tissue.
2. The activity of this enzyme in adipose tissue from obese persons is about three times as high as that from non-obese persons.
3. Following hospital treatment including a restricted diet especially low in carbohydrates the phosphorylase activity declines to normal values.
4. The findings are interpreted as a manifestation of enzymatic adaptation.

The experiments here reported have been published in detail in *Reports of the Steno Memorial Hospital and the Nordisk Insulinlaboratorium* 1953, *5:* 30.

References:

Arndt, H. J.: Ziegler's Beitr. z. pathol. Anat. 1927, *79:* 69, 523.
Cori, G. T. & C. F. Cori: J. Biol. Chem. 1940, *135:* 733.
Engel, F. L.: Endocrinology 1951, *49:* 127.
Engel, F. L. & J. L. Scott, Jr.: Endocrinology 1950, *46:* 574.
Idem: Endocrinology 1951, *48:* 56.
Green, A. A. & G. T. Cori: J. Biol. Chem. 1943, *151:* 21.
Haagensen, N. R.: Reports of the Steno Mem. Hosp. and the Nordisk Insulinlaboratorium 1953, *5:* 7.
Mandelstam, J. & J. Yudkin: Biochem. J. 1952, *51:* 681.
Martin, J. B. & D. M. Doty: Anal. Chem. 1949, *21:* 966.
Mirski, A.: Biochem. J. 1942, *36:* 232.
Renold, A. E., A. Marble & D. W. Fawcett: Endocrinology 1950, *46:* 55.
Schoenheimer, R.: The Dynamic State of Body Constituents, Cambridge, Harvard Univ. Press 1942.
Shapiro, B. & E. Wertheimer: Biochem. J. 1943, *37:* 397.
Spiegelman, S.: Modern aspects of enzymatic adaptation. J. B. Sumner & K. Myrbäck: The Enzymes, N. Y. 1950, p. 300.
Stetten, D. Jr. & B. V. Klein: J. Biol. Chem. 1945, *159:* 593.
Tuerkischer, E. & E. Wertheimer: J. Physiol. 1942, *100:* 385.

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